DISSERTATION ON

ROLE OF MASPIN EXPRESSION IN ORAL SQUAMOUS CELL CARCINOMA

Dissertation submitted to

THE TAMILNADUDr.M.G.R. MEDICAL UNIVERSITY,
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APRIL 2018 EXAMINATIONS

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The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 25.10.2016 at the Council Hall, Stanley Medical College, Chennai-1 at 2PM

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Dr.K.SARANYA

ABBREVIATIONS

SCC : Squamous Cell Carcinoma

HNSCC : Head and Neck Squamous Cell Carcinoma

COX-2 Cyclooxygenase -2

WHO : World Health Organisation

RMT : Retromolar Trigone

HPV : Human Papilloma Virus

OSCC : Oral Squamous Cell Carcinoma

URT : Upper Respiratory Tract

WD : Well differentiated

MD : Moderately differentiated

PD : Poorly differentiated

IHC : Immunohistochemistry

H & E : Hematoxylin & Eosin

IARC : International Agency for Research on Cancer

DNA : Deoxyribo Nucleic Acid

CT : Computed Tomography

MRI : Magnetic Resonance Imaging

USG : UltraSonoGram

PET : Positron Emission Tomography

TNM : Tumor, Node, Metastasis

PGP 9.5 : Protein Gene Product 9.5

HPE : Histopathological Examination

OME : Over All Maspin Expression

Urkund Analysis Result

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INTRODUCTION

Oral cancers broadly includes neoplasms arising from buccal mucosa , tongue , lips , hard palate , soft palate , oropharynx , larynx , hypopharynx except the neoplasm arising from salaivary gland. Among this 90% comprises squamous cell carcinoma.

Oral cancer is the most common cancer in India among men (11.28% of all cancer) and fifth among women (4.3% of all cancers) and 3rd most frequently occurring cancer in India among both men and women [(1)]. Oral SCC is the 6th leading cause of cancer worldwide. [(2)]

Squamous cell carcinoma arises from the lining epithelium of oral mucosa . Oral SCC is a more aggressive tumour and it has increased tendency to spread to cervical lymphnodes. Nodal metastasis also determines the prognosis and survival outcome of the patient.

It is a multistep carcinogenesis process involving numerous oncogenes and tumour suppressor gene. Because of increased incidence and high recurrence rate with increased morbidity and mortality, it is mandatory to understand the neoplastic transformation at sub cellular level[(3)].

Now many studies are going on to establish the various prognostic factors for oral SCC. Especially at the molecular level including p53 various tumour suppressor gene has been identified in the etiology of oral squamous cell carcinoma [(4)].

MASPIN – Mammary Serine Protease Inhibitor belongs to serine protease inhibitor family. Proteases which breaks down extracellular matrix and promotes tumour invasion and metastasis . But maspin being a proteases inhibitor which prevents metastasis and tumour progression . MASPIN also induces apoptosis of the tumour cells by the activation of CASPASE pathway[5]. MASPIN has various implication in oral SCC not only in the form of tumour progression and metastasis but it also has role in tumorigenesis and differentition .

MASPIN also has its role in endothelial apoptosis (ie) antiangiogenic property. So targetted therapies are established against MASPIN to control tumour angiogenesis and metastasis by that reduces the tumour progession [6].

The main purpose of this study is to establish the interrelation ship between clinicopathological findings and MASPIN expression and its prognostic impact.

AIMS AND OBJECTIVES

1) To study the incidence of oral SCC in patients admitted in Govt.Stanley Medical College , Chennai during the period of January 2016 to December 2016

2) To identify the incidence of oral SCC in various sites.

3) To study the clinicopathological features and various prognostic factors of oral SCC including age , sex, risk factors , histological grade , tumour staging and nodal metastasis.

4) To determine the immunohistochemical expression of MASPIN.

5) To evaluate the level of MASPIN expression in various grades of oral SCC.

6) To compare the overall MASPIN expression (OME) with various prognostic factors.

REVIEW OF LITERATURE

EPIDEMIOLOGY :

Oral SCC ranks 6th among all cancers worldwide[7] . It can arises from any part oral cavity but most commonly affected site is tongue ,floor of mouth[11]. It ususally arises from premalignant lesions.

There are various risk factors involved in origin of SCC among that tobacco chewing ,betal nut chewing ,alcohol is considered as most important risk factor[8].

Oral SCC occurs most common among men than in women because men are most commonly exposed to risk factors such as moking , betal nut chewing and alcohol intake. The development of SCC also depends on the age and duration of exposure [9] .

As age increases age related mutagenic and epigenetic changes occurs. Various syndromes are associated with oral SCC namely Li Fraummeni syndrome , Plummer Vinsons Syndrome, Fanconi's anaemia , Dyskeratosis Congenita, Xeroderma Pigmentosa, Discoid Lupus Erythematosus, chemotherapy induced immunosupression of organ transplant[10] .

The most common site involved is ventral surface of tongue and floor of mouth[11] . The reason behind this is it is lined by non keratinized squamous epithelium. Since it is non keratinised the carcinogens easily penetrate , accumulate and affects the progenitor cells .These carcinogens constantly accumulate and bathe the tissues of floor of the mouth and tongue.

ANATOMY:

Head and neck is the most complex part of our body

ORAL CAVITY: The oral cavity is a multifaceted organization tailored to perform various functions including mastication, ingestion, taste sensation, immune surveillance and speech.

Oral cavity comprises of the roof, floor and lateral walls. The roof separates the oral cavity from nasal cavity by hard palate and soft palate. Floor is formed by the muscular diaphragm- mylohyoid muscle. The lateral Walls or cheek are formed by the muscle lined by mucosal layer. The cheek continues anteriorly as lips called as anterior fissure and posteriorly into Oropharynx. This opens into pharynx. Soft palate and tongue surrounds the oropharynx.

COMPONENTS:

It can be divided into eight sub sites:

1. Lip
2. Buccal mucosa
3. Lower alveolar ridge

4. Upper alveolar ridge

5. Retro molar trigone (retro molar gingiva)

6. Hard palate

7. Anterior 2/3 of tongue and

8. Floor of the mouth.

FLOOR OF THE MOUTH:

It is a horse shoe – shaped mucosal area between the gingiva of the lower alveolar ridge laterally or ventrally and the lateral border of the tongue medially, extending dorsally to the left and right tonsillar areas. The frenulum of the tongue which divides the tongue into right and left sides. It contains ostia of the sublingual and submandibular salivary glands.

HARD PALATE:

The roof of the oral cavity is formed by hard palate formed by portions of maxillary and palatine bones.

RETRO MOLAR TRIGONE:

It is a triangular mucosal surface that lined the ventral surface of the ascending mandibular ramus.

MICROANATOMY OF ORAL MUCOSA:

The squamous epithelium lining the oral mucosa is composed of keratinocytes which is stratified. Basal cells are the one that helps to maintain the normal thickness of the epithelium by their constant replication. The basal cells are constituted by the organized units of stem cells and transit amplifying cells. The transit amplifying cells divide frequently in short intervals whereas the stem cells divide infrequently.

The oral squamous epithelium has a longer turn over time when compared with gastrointestinal mucosa . It takes about 25 days for buccal epithelium and 50 days for gingival epithelium[(12)]. Lamina propria that lies beneath the epithelium is composed of fibrous tissue with very rich neurovascular supply.

The crucial factor for the homeostatic maintenance of oral mucosa is the interface between the epithelium and lamina propria.

VARIATION IN THE LINING EPITHELIUM IN ORAL SITES

Epithelial type	**Thickness**	**Site**
Orthokeratinised	Thick	Hard palate,gingiva
Parakeratinised	Thick	Gingival,dorsal Tongue, alveolar mucosa
Non keratinised	Thick	Buccal and labial mucosa

Non keratinised	Thin	Ventrolateral tongue,floor of the mouth,soft palate and gingival sulcus

ORAL EPITHELIAL CELL KINETICS

The oral epithelial cell proliferation index is the one exhibiting high labelling index rate of 11.7% in non keratinized lining mucosa than the thick keratinized gingival mucosa, which showed an index of 8.5%[13,14].

NASAL CAVITY:

Nasal cavity forms the uppermost part of the respiratory tract. It is formed of two nasal cavities which open into nares anteriorly, choanae posteriorly and limited laterally by orbits.

PHARYNX:

The link between oral cavity and nasal cavities with larynx and esophagus in the neck is established by musculofascial half cylinder. They comprise nasopharynx, oropharynx and laryngopharynx. Retropharyngeal space is the loose connective tissue zone which separates pharynx from vertebral column. Skeletal muscle and fascia forms the pharyngeal wall.

NASOPHARYNX:

The nasopharynx is the part formed between posterior apertures of nasal cavities or choanae and the level above soft palate. The roof and lateral walls are formed by domed vault of pharyngeal cavity which consists of sphenoid bone and basal parts of occipital bone.

OROPHARYNX:

Posterior opening of oral cavity is known as oropharynx. It is bound between inferior level of soft palate and upper part of epiglottis. The oropharynx is bound by posterior one third of tongue and lingual tonsil anteriorly , palatoglossal and palatopharyngeus muscle on both sides.

TONSILS:

The largest collection of lymphoid tissue in the oropharynx is known as palatine tonsil. The lymphoid collection in the roof of nasopharynx is called as adenoids or pharyngeal tonsils. Lingual tonsil is present in posterior one third of tongue.

LARYNGOPHARYNX:

Laryngopharynx is bound between the margin of epiglottis and superior part of esophagus. It comprises two important anatomical landmarks. Anteriorly it forms mucosal pouches called as valleculae. The other one is pyriform fossa between the central part of larynx to lateral part of thyroid.

LARYNX:

Larynx forms the upper airway tract which continues as trachea inferiorly forming the lower respiratory tract. It has three large unpaired cartilages, three smaller cartilages,

fibroelastic membrane and numerous intrinsic muscles. It is a tubular structure lined by mucosa formed by superior margin of epiglottis anteriorly, aryepiglottic fold laterally and interarytenoid notch posteriorly.

LYMPHATIC DRAINAGE OF ORAL CAVITY:

The following are the three groups of nodes that are mainly involved:

a. Jugulodigastric lymphnodes
b. Submental lymphnodes
c. Submandibular lymph nodes

- Gingival lymph vessels drain to the submandibular lymph nodes, while those from lower incisor region drain to the submental nodes.

- Vessels from palate mostly are drained by jugulodigastric lymph nodes, but some are drained by retropharyngeal nodes.

- The rich lymphatic plexus in the lateral third of dorsum, lateral border, and part of ventral part of tongue drain into the ipsilateral submandibular lymph nodes.

- Central lymph vessels run to bilateral submandibular nodes; some directly run to the jugulo-omohyoid nodes.

- Jugulodigastric, Jugulo-omohyoid or the intermediate nodes unilaterally or bilaterally drain the lymphatics vessels from circumvallate papillae.

ETIOLOGY :

The etiology of precancerous and cancerous lesions of oral and upper respiratory tract infection are multifactorial. The most common risk factor is tobacco use, smoking, snuffing or chewing , betal quid and high alcohol intake . The risk of oral SCC increases to 80% by the usuage of tobacco and alcohol [(20)].

TOBACCO:

In India tobacco chewing is considered as main risk factor for oral and oropharyngeal malignancies in about 50%in men and about 90% of women. The risk intensifies and it is three and half times higher in smokers when compared to non smokers [(15 ,21)]. It may be used in various forms like snuff dipping , chewing , smoking with cigars , beedies and pipes.

IARC confined that tobacco contains more than 70 carcinogens.The most common among them is polycyclic hydrocarbons and nitrosamines[(16)]. The cytochrome p450 oxidising enzyme is responsible for the formation of reactive carcinogenic intermediates. If these carcinogens fail to get detoxified , it forms the adducts between the carcinogenic agents and the keratinocyte DNA[(17)].

ARECA NUT :

Few studies also shown that areca nut which contains calcium hydroxide when it combines with tobacco it increases the relative risk to 8.15 times as their risk is 1- 4 times without tobacco. When chewing it releases reactive oxygen species that induces mutation[(22)].

SMOKING :

Smokers are more prone to develop oral and upper respiratory tract malignancies when compared to non smokers and the risk is three and half times more in smokers .The risk is determined by the intensity and duration of smoking more than 20 cigarretes/day for more than 20 years duration increases the risk of malignancy and also cessation of smoking decrease the risk of malignancy to three quarters . Smoking increases the prostaglandin levels and also it overexpresses various genes responsible for carcinogenesis [18, 23].

ALCOHOL:

Important carcinogen present in alcohol are nitrosamine, acrylide and polyphenols[19].

Ethanol when metabolized to acetaldehyde becomes mutagenic and act along with tobacco smoking in the oral carcinogenesis. Alcohol act as solvents and increases the permiability of carcinogens to oral mucosa[24].

INFECTION :

Infection with HPV plays a vital role in a subset of Head and Neck cancers[25,26,27]. There is an increasing incidence in HPV associated oropharyngeal SCC [28] . HPV was detected more frequently in oropharyngeal SCC's (in tonsils, base of tongue) in comparison with cancers of oral cavity or other HNSCC subsites[26,27,29].

Though more than 150 serotypes of HPV are present, infection with high risk HPV types 16 and 18 and low risk HPV types 6 and 11 only are associated with 20-25%

of Head and neck cancers particularly oral and oropharyngeal cancers[28].**The most prevalent type in HPV-positive oral cancers is HPV type16 (Over90%)** [29,30,31].

HPV positive cancers are clinically distinct from HPV negative cancers[32,33,34]. Oral HPV infection is transmitted sexually or perinatally. Tumors of oropharynx particularly tonsillar tissue are more likely to be associated with HPV. The risk of oral HPV infection is linked to sexual behaviors such as oral sex, oro-genital sex and increased number of sexual partners. People with HPV associated cancers are younger, less likely to be smoker and drinkers but with different sexual behavior and marijuana use [32,33,34].

GERD : It is also considered as a risk factor for laryngeal carcinoma.

OTHER FACTORS :

Factors considered in developing oral cancer and its progression includes ultraviolet irradiation , poor oral hygiene, immune suppression, periodontal disease ,trauma, dental irritation , Xeroderma pigmentosa , Fanconis anaemia, Blooms syndrome[10,35].

PREVENTIVE FACTORS :

Vitamin A,C ,E are antioxidants which have protective role in oral cancer. 90% of oral cancer can be prevented by consumption of fruits and vegetables[36,24]. Iron maintains the thickness of the oral epithelium . Deficiency of iron causes oral atrophy leading to cancer in upper air and food passages.

Vaccines developed against HPV infection also have protective effect against oral and upper respiratory tract SCC.

CLINICAL FEATURES OF ORAL AND OROPHARYNGEAL SCC :

The patients with oral and oropharyngeal cancers are usually asymptomatic or can presents with vague symptoms. Patients with high risk behavior usually presents with red or white plaques. Advanced disease presents as proliferative growth or as nodules. The patients present with symptoms like referred pain to ear , difficulty in speaking ,swallowing, bleeding and weight loss. Such patients can also presents with neck swelling (cervical lymphnodal secondaries). Advanced cases may also present with orocutaneous fistula.[38]

CLINICAL FEATURES OF UPPER RESPIRATORY TRACT SCC:

Like oral cavity , it also presents as a proliferative growth or as plaques or as ulcers. The symptomatology vary according to the site. Patients can present with blood tinged postnasal drip , head ache, or as serous otitis media. But 10% of cases are asymptomatic.

Glottic tumours presents as hoarseness of voice. Supraglottic tumours and hypopharyngeal tumours presents with voice change, neck swelling, haemoptysis and difficulty in swallowing. The subglottic tumours present with difficulty in breathing and stridor [37,38].

DIAGNOSTIC PROCEDURES:

NON INVASIVE PROCEDURES:

Invasive procedures are always preceded by imaging modalities. MRI is the modality of choice than CT it is superior in assessing soft tissue infiltration, bone erosion, loco regional metastasis and intracranial metastasis particularly in cases of nasopharyngeal carcinoma [39,40].

Other imaging modalities includes X-ray or CT – chest, bone scan, positron emission tomography with CT (PET-CT) and USG/CT- Abdomen for work up .

INVASIVE PROCEDURES :

All lesions are first proceeded with endoscopy followed by biopsy . Biopsies are easily taken from the grossly visible lesions. If it is not visible grossly then multiple biopsies are taken from suspicious sites.

Usually incisional biopsies are performed for all lesions but major issue with this is adequate size and depth including lamina propria is difficult in thick keratinising lesions. Punch biopsy is preferred because it gives good yield of tissue and produce minimal trauma [41]. A proper orientation of the specimen is important otherwise it can exaggerate the complex architecture in the interphase between the oral epithelium and the lamina propria. In laser excision technique the entire potentially malignant lesion can be sampled.

Other methods :

1) DNA CYTOMETRY[42]
2) EXFOLIATIVE CYTOLOGY
3) FLUORESCENCE IMAGING

Exfoliative cytology has high sensitivity and specificity in diagnosing premalignant lesions and dysplasias. Dysplasias should always be proceded with biopsy as invasion is not made out in exfoliative cytology. The combination of exfoliative cytology and DNA cytometry analysis in patients with suspicious lesion aids in diagnosing the malignancy, 15 months prior to histological confirmation[42,43].

MOLECULAR PATHOGENESIS OF ORAL SQUAMOUS CELL CARCINOMA:

Development of oral squamous cell carcinoma involves multiple factors including genetic alterations and acquired causes like alcohol consumption, smoking, microorganisms , chemical carcinogens and ultraviolet and ionisisng radiations(36).

Oncogenes are activated through gene amplification, augmented transcription or increased transforming activity due to various mutations . The inactivation of tumour suppressor genes occurs through various genetic changes such as mutation, deletion , loss of heterozygosity or by epigenetic alterations mainly DNA methylation or chromatin alteration[36].

FIELD CANCERISATION :

The theory as defined by field cancerisation states that the entire oral epithelium is at risk of developing malignancy as a result of constant exposure to several carcinogenic factors and accumulation of genetic aberrations affecting the oncogenes and tumor suppressor genes[44].

According to this theory various oral cancers develop from independent cell clones. More recent studies modified this theory into the **patch field carcinoma model (fig 1).**[45,46] This model states that stem cells located in the basal layer of oral epithelium acquires a genetic aberration which is transferred to its daughter cells. This patch of cells expand and cannot be seen macroscopically. In some instance it may present clinically as either leukoplakia or erythroplakia.

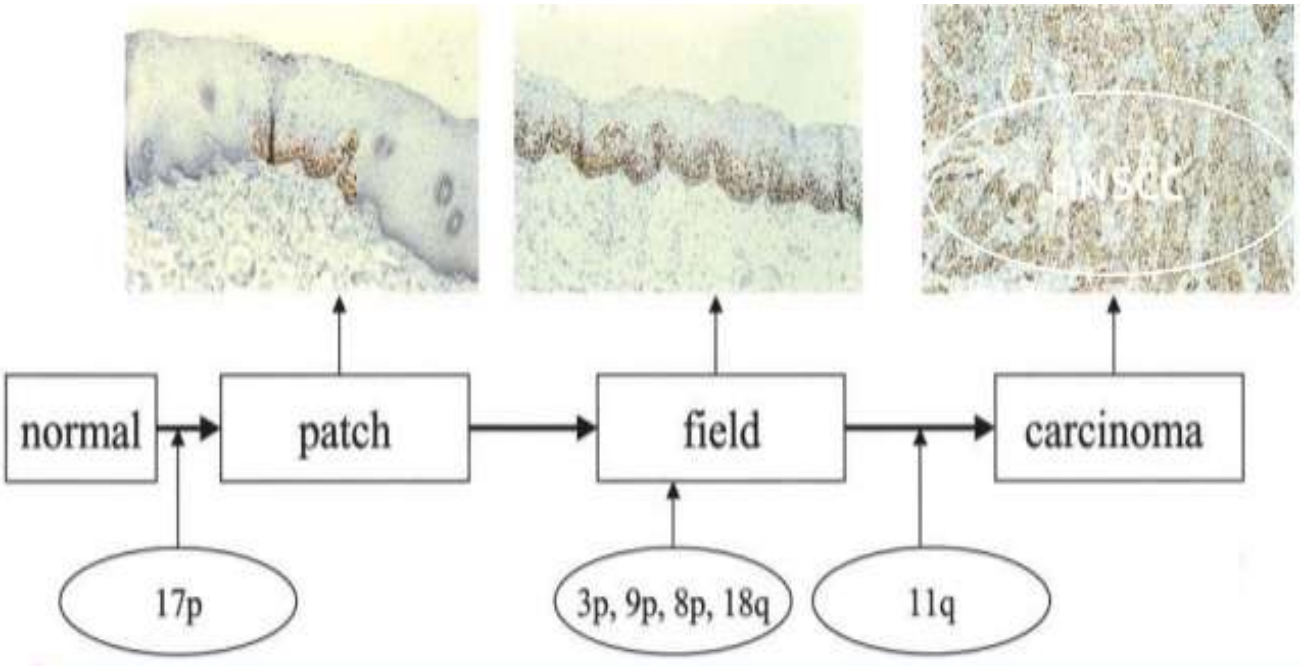


Figure 1 : PATCH FIELD CANCERISATION MODEL

Classification of oral potentially malignant disorders:

Precancerous lesions:

- Leukoplakia
- Erythroplakia
- Erythroleukoplakia

Pre cancerous conditions:

- Oral submucosal fibrosis
- Actinic keratosis
- Lichen planus
- Siderophagic dysphagia
- Discoid lupus erythematous
- Palatal lesions in reverse cigar smoking
- Syphilis
- Dyskeratosis congenital
- Epidermolysis bullosa

PREMALIGNANT LESIONS OF ORAL SQUAMOUS CELL CARCINOMA:

Premalignant lesions are defined by WHO as "A morphologically altered tissue in which cancer is more likely to occur than in its apparently normal counterpart"[47].

Squamous mucosa of oral cavity, oropharynx and upper aerodigestive tract are subjected to various carcinogenic stimuli in the mode of chronic irritation, trauma , infection and carcinogenic exposure. This can present as red or white plaques called erythroplakia or leukoplakia respectively.

A study by Mashberg and Fieldman et al, have demonstrated the transformation of 90% cases of erythroplakia into severe dysplasia and infiltrating squamous cell carcinoma (37%)[48]. In leukoplakia transformation into invasive squamous cell carcinoma is observed in 8% of cases. Thereby, erythroplakia has been demonstrated as having highest malignant potential.

LEUKOPLAKIA

The term leukoplakia was coined by Schwimmer Budapest in the year 1877 [49] . It presents as a white plaque like lesions . More common among smokers with higher predilection for floor of mouth. In non smokers it is most commonly involves lateral border of the tongue[50] .

Histologically , it presents as epithelial atrophy , dyskeratosis hyperplasia with or without hyperkeratosis, different grades of dysplasia or carcinoma in situ[51] (fig .2)

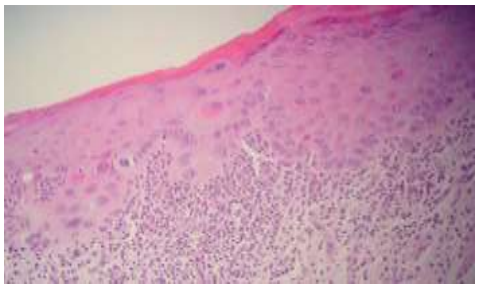
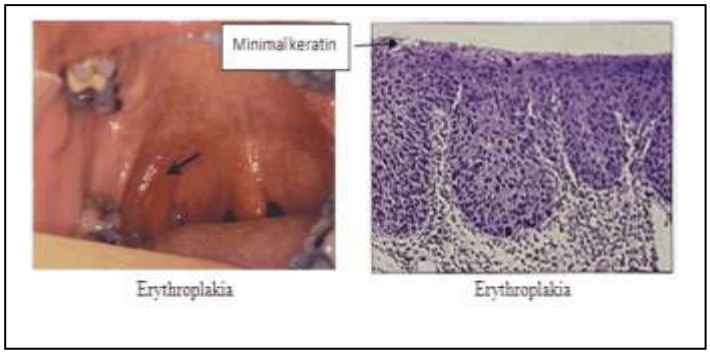


Figure 2 : HPE OF LEUKOPLAKIA

ERYTHROPLAKIA :

It presents as a red velvety patches . Histologically it presents as atrophy or varying grades of dysplasias and carcinoma in situ.



ERYTHROLEUKOPLAKIA :

Erythroplakic changes developing in leukoplakic nodules are called as eythroleukoplakia . Associated with candidal infection . It is associated with higher malignant transformation when compared to leukoplakia alone.

SQUAMOUS CELL CARCINOMA :

According to WHO , Squamous Cell Carcinoma is defined as epithelial neoplasm with varying degree of differentiation and increased propensity for lymph nodal metastasis.

WHO CLASSIFICATION USED

FOR ORAL CAVITY (ANNEXURE –I)

FOR OROPHARYNX AND NASOPHARYNX (ANNEXURE II)

FOR HYPOPHARYNX AND LARYNGEAL CARCINOMA (ANNEXURE-III)

HISTIOGENESIS:

Squamous cell carcinoma arises from mucosa where squamous epithelium form its lining and from ciliated columnar epithelium where it undergone squamous metaplastic changes like in larynx[52].

Conventional squamous cell carcinoma :

Conventional squamous cell carcinoma can be classified into keratinizing and non keratinizing type . The most common is keratinizing type.

Depending on the keratin pearl formation , degree of differentiation it is further classified into

- Well Differentiated ,
- Moderately Differentiated And
- Poorly Differentiated Squamous Cell Carcinoma.
    - **Well Differentiated Squamous Cell Carcinoma** : It has features of individual cell keratinisation , keratin pearl formation, mild to moderate hyperchroamtic pleomorphic nuclei with mild degree of mitotic activity.
    - **Moderately Differentiated Squamous Cell Carcinoma** : It has features of less keratinisation , distinct nuclear atypia and mitotic activity.
    - **Poorly Differentiated Squamous Cell Carcinoma**: It presents with marked atypia with immature cells , increased mitotic activity with less features of keratinisation .

Invasiveness of squamous cell carcinoma is of either diffuse spreading or of pushing type . Cells in diffuse type spread as single cells or in cords . It has propensity for lymhovascular invasion . It has very less survival rate of 30-40%.

Pushing type has good survival of 80-90% because it presents with less lymphovascular invasion .A study conducted by Crissman and Zarbo et al discussed the correlation of prognosis with invasive pattern[53]

Stroma shows desmoplastic reaction because of deposition of extracellular matrix , collagen and myofibroblast . There is foreign body reaction to keratin with formation of granuloma and chronic inflammatory cell formation is also common..

Dysplatic changes is common in epithelium adjacent to carcinoma.

VARIANTS OF SQUAMOUS CELL CARCINOMA :

- **VERRUCOUS CARCINOMA** :

It is a well differentiated form of squamous cell carcinoma . It is otherwise called as **ACKERMANN TUMOUR** .The most common sites are oral cavity and larynx. Risk factor associated with this is tobacco smoking and HPV.

Macroscopically these are locally aggressive slow growing neoplasm with warty exophytic lesions with pushing margins.

Microscopically the tumour has club shapped papillae with mature squamous cell proliferation .The surface of the epithelium showing church spire keratosis.The biopsy sample should include stroma .Otherwise biopsy sample is considered as inadequate. It has very good prognosis.

- **BASALOID SQUAMOUS CELL CARCINOMA:**

It is a rare but most aggressive variant. Most common site is pyrifrom fossa. The most common risk factor associated is tobacco , alcohol consumption and HPV infectoion . It is presents as an ulcerated mass with induration of submucosa. Microscopically it is composed of both basaloid cells and squamous cells with cystic spaces and comedo necrosis seen. Early recurrence and local metastasis are the features of Basaloid SCC.

- **ACANTHOLYTIC SQUAMOUS CELL CARCINOMA:**

It is a rare variant.it is more common among sun exposed areas. Most common site are supraglottic larynx and hypopharynx. There is no specific risk factor related to it. It is composed of acantholytic squamous cells with pseudo lumina formation . The main differential diagnosis is angiosarcoma. The prognosis is same as that of conventional SCC .

- **PAPILLARY SCC** :

It is otherwise known as exophytic SCC. Most common site is larynx and hypopharynx. Grossly it presents as a polypoidal friable mass with broad base . Microscopically , they presents with malignant cells arranged in polypoidal pattern with fibrovascular core.The tumour cells are highly pleomorphic with invasion into stroma .The precursor lesions are papilloma and mucosal hyperplasias. The prognosis is good.

- **SPINDLE CELLS SCC:**

Most common site is laynx. It is a biphasic tumour composed of both conventional SCC and spindle cell component invading into underlying stroma. It arises from epithelial but mimicks like mesenchymal origin. The most common risk factor is radiation exposure. Macroscopically it presents as a polypoidal mass with thin pedicle. Sometime it may autoamputate and is expectorated in the sputum. Microscopically it is composed of biphasic population of cells with both malignant squamous cells and malignant spindle cells.It has poor prognosis.

- **ADENOSQUAMOUS VARIANT OF SCC** :

It is a high grade tumour and the most common site being larynx and hypopharynx. It originates from totipotent cells in the basal region. It has poor prognosis. It is composed of both adeno and squamous cell component with mucin positivity in the mucin component.

- **LYMPHOEPITHELAIL CARCINOMA** :

It is an undifferentiated carcinoma with extensive lymphocytic infiltrate. It carries poor prognosis with common site being larynx and hypophaynx.

- **GIANT CELL CARCINOMA:**

It is an undifferentiated carcinoma with multiple multinucleated giant cells with neutrophils and cell debris in its cytoplasm .It carries a poor prognosis. Common sites are larynx and hypopharynx.

STAGING :

Prognostic evaluation is based on TNM classification. Two well established factors such as **TUMOUR THICKNESS AND EXTRACAPSULAR NODAL SPREAD** are considered as important in determining the behaviour .

GRADING :

According to WHO grading system 3 categories are recommended

- Well differentiated,
- Moderately differentiated and
- Poorly differentiated.

It depends on the subjective assessment of keratinisation, pleomorphism and mitotic activity.

BRODER'S GRADING SYSTEM[54]:

- Grade I : Well differentiated- <25% of undifferentiated cells
- Grade II : moderatelydifferentiated -<50% of undifferentiated cells
- Grade III : Poorly differentiated- <75% of undifferentiated cells
- Grade IV : Anaplastic or pleomorphic->75% of undifferentiated cells

ANNEROTH'S GRADING :

It includes the six parameters of which 3 are connected to tumor population and other 3 are connected with tumor host relationship. The six parameters of Anneroth's histological grading system included

- Degree of keratinitization,

- Nuclear pleomorphism,
- Number of mitoses,
- Pattern of invasion,
- Stage of invasion,
- Lympho-plasmocytic Infiltration.

Morphological parameter	**Points**			
	1	2	3	4
Degree of keratinisation	Highly keratinised (50% of the cells)	Moderately keratinised (20-50%of the cells)	Minimally keratinised (5-20%of the cells)	No keratinisation (0-50%of the cells)
Nuclear pleomorphism	Little nuclear pleomorphism(75% mature cells)	Moderately abundant nuclear pleomorphism(50-75%mature cells)	Abundant nuclear pleopmorphism(25-50% mature cells)	Extreme nuclear pleomorphism (0-25%mature cells)
No.of mitosis/HPF	0-1	2-3	4-5	5

The histological pattern often reflects the metastatic potential and is correlated with the survival.

Prognostic factors :

- **Site :**

Lip is the most common site followed by anterior tongue , floor of mouth , glottis, supraglottis, posterior part of tongue ,sub glottis, hard palate and soft palate.[59]

- **Stage:**

It is the significant parameter predicting the prognosis. The recurrence-free 5-year survival rates for stage I, 91.0%; stage II, 77.2%; stage III, 61.2%; stage IVA, 32.4%; stage IVB, 25.3%; stage IVC, 3.6%.[56,60].

- **Grade:**

Grading of the deep invasive margins is more important than grading of the entire tumour in predicting the prognosis.[55,61]

- **Depth of invasion:**

This is an important prognostic factor included in the staging system.[62]

- **Size:**

The clinical outcome is not dependent on the size of the tumour except for the small sized tumour.[63]

- **Desmoplastic response:**

In lip carcinoma, presence of desmoplasia is a predictor of aggressive behavior.[64]

- **Tissue eosinophilia:**

Eosinophilic infiltration indicates a better Prognosis[57,65].

- **Lymph node involvement:**

It is a key feature in the staging system. Presence of extra capsular spread is an indicator of decreased survival rate.[66]

- **DNA ploidy:**

It correlates with tumour grade and an independent prognostic factor with nondiploid tumors carrying an unfavourable prognosis.[67]

- **HPV 16:**

Presence of this is an indicator of improved survival among patients with oropharyngeal carcinoma.[58]

P16:

It is a surrogate marker of high risk HPV which carries a favourable Prognosis[68].

IMMUNOHISTOCHEMISTRY:

Albert Coons et al in 1941 first labelled antibodies directly with Fluorescent isocyanate. Nakane and Pierce et al, in 1966, introduced the indirect Labeling technique in which the unlabelled antibody is followed by second Antibody or substrate. Various stages of development of immunohistochemistry Include peroxidase – antiperoxidase method (1970), alkaline phosphatase Labeling (1971), avidin-biotin method (1977) and two layer dextrin polymer Technique (1993).

Immunohistochemisty involves TWO PRINCIPLES immunology and histology.

IHC is used to determine the particular antigen and its subcellular location . IHC uses antibodies to detect antigenic differences between the cells. These differences help us to specifically identify the lineage of cell populations and define biologically distinct population of cells within the same lineage.

The use of antiboby in IHC depends on sensitivity and specificity of antigen antibody reaction as well as on the hybridoma technique which provides limitless source of highly specific antibodies

ANTIGEN RETRIEVAL:

The following techniques are used for antigen retrival

1. Proteolytic enzyme digestion
2. Microwave antigen retrieval
3. Pressure cooker antigen retrieval
4. Microwave and trypsin antigen retrieval

PROTEOLYTIC ENZYME DIGESTION :

The enzyme used is trypsin and anti proteinase. It breaks down the formalin cross linkages and unmask the antigen determitants.

Disadvantages include overdigestion , underdigestion and antigen destruction .

MICROWAVE ANTIGEN RETRIEVAL:

This is a newer technique most commonly used in current practice. Microwave oven heating involves boiling formalin fixed paraffin sections in various buffers for rapid and uniform heating.

PRESSURE COOKER ANTIGEN RETRIEVAL:

Miller et al in 1995 compared and proved that pressure cooking method has fewer inconsistencies, less time consuming and can be used to retrieve large number of slides than the microwave method .

PITFALLS OF HEAT PRETREATMENT:

Drying of sections at any stage after heat pretreatment destroys antigenicity. Nuclear details are damaged in poorly fixed tissues. Fibers and fatty tissues tend to detach from slides while heating. Not all antigens are retrieved by heat pretreatment and also some antigens like PGP 9.5 show altered staining pattern.

DETECTION SYSTEMS:

After the addition of specific antibodies to antigens, the next step is to visualize the antigen-antibody reaction complex. The methods employed are direct and indirect methods.

In the direct method, primary antibody is directly conjugated with the label. Most commonly used labels are fluorochrome, horse radish peroxidase
and alkaline phosphatase.

Indirect method is a two-step method in which labelled secondary antibody reacts with primary antibody bound to specific antigen. The use of peroxidase enzyme complex or avidin-biotin complex further increases the sensitivity of immunohistochemical stains

In 1993, Pluzek et al introduced enhanced polymer one step staining, in which large numbers of primary antibody and peroxidase enzymes are attached to dextran polymer back bone. This is the rapid and sensitive method. Dextran polymer conjugate two step visualization system is based on dextran technology in Epos system. This method has greater sensitivity and is less time consuming.

Molecular pathogenesis and genetics of oral squamous cell carcinoma:

Oral carcinogenesis is a progressive disease and normal epithelium passes through stages starting from dysplasia to finally transforming into invasive phenotypes.

Genetic alteration in oncogenes or tumour suppressor genes ,genomic instability and epigenetic modifications are involved in oral carcinogenesis.

Several oncogenes are implicated in the oral carcinogenesis. Aberrant expression of EGFR, k-ras, c-myc and bcl are involved in oral carcinogenesis. TGF- beta promotes neovascularization and mitogenesis.(92)

Several tumour suppressor genes are also involved in the carcinogenesis of oral scc. Among that p53 and p16 plays an important role.

Maspin a tumour suppressor gene is involved in carcinogenesis of oral SCC . It acts as a main prognostic factor by preventing invasion ,angiogenesis and metastasis

MASPIN (MAMMARY SERINE PROTEASE INHIBITOR) :

- It belongs to serine protease inhibitor superfamily coded by serpin b5 gene located on 18q21.3-q23 and was first identified in1994[(69)].
- It is a tumour suppressor gene and prevents the invasion ,angiogenesis and progression of neoplastic cells.
- It is expressed in skin , prostate, breast ,testis, intestine ,tongue ,lung and thymus.
- It is down regulated in breast ,prostate , gastric carcinomas and melanomas.
- It is located subcellulary at cytoplasm, nucleus. It is also present in the extracellular region ,extracellular exosome and in extracellular spaces[(83,84,85,86)].

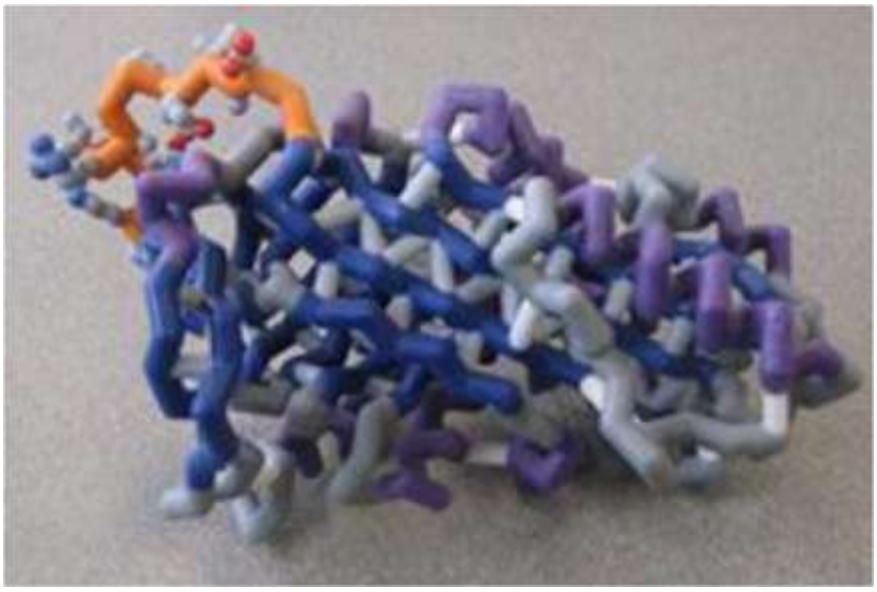


Figure 3: **CRYSTALLOGRAPHIC PICTURE OF MASPIN**

Basic functions(74,75) :

1. Morphogenesis of an epithelium
2. Regulation of epithelial cell proliferation
3. Extracellular matrix organization
4. Negative regulation of endopeptidase activity
5. Regulating the breakdown of proteins by Inhibiting the catalytic activity of proteinases
6. It also regulates phagocytosis,coagulation and fibrinolysis.

Molecular level activity :

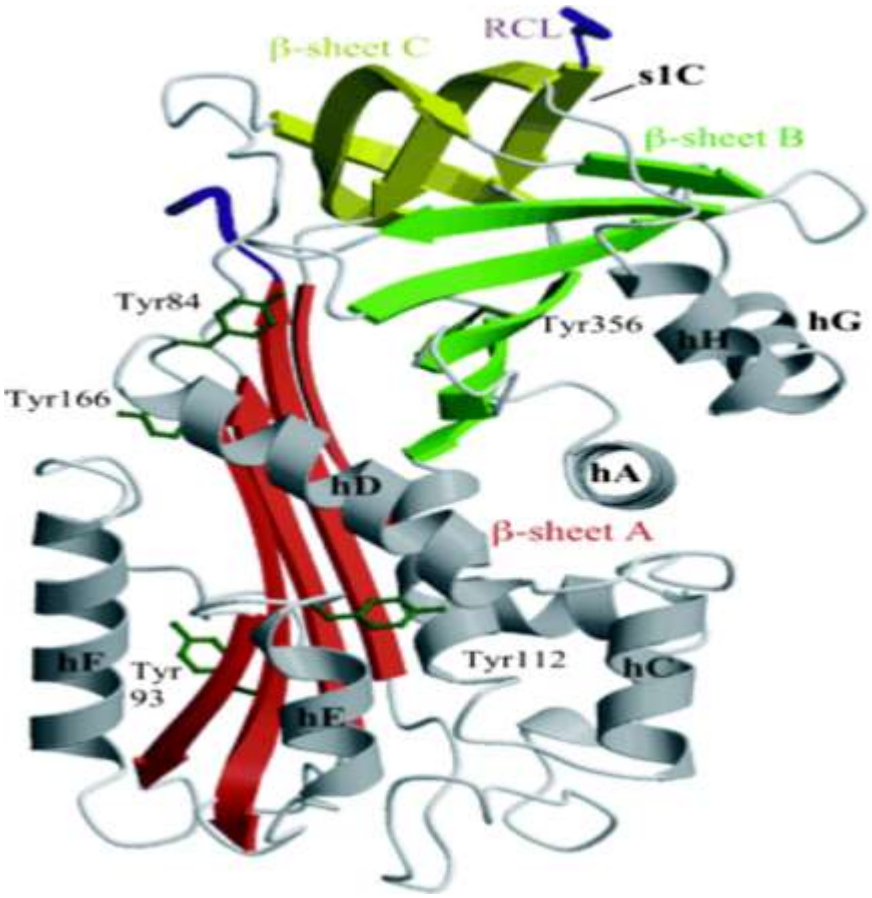
Serpin exhibits its function by conformational change from stress to relaxed state .

RSL – reactive site loop is the key component in the serpin family[(76)].

It is located in 9-15 residues amino terminal to the reactive site peptide bond. This allows the reactive site to present an optimal configuration for binding and inhibiting target protease.

Stressed Catalytic serine residue in the protease , changes the conformation of the RSL loop to form an acyl intermediate . Conformational change to the relaxed state levels to irreversibly trapping of the protease in an active state [(77,78,79)].

Thus serpin functions as a suicide inhibitor of the protease.



MASPIN also act as an inhibitor of histone deacytylase.

Its function, as serine protease inhibitor is down regulated in cancers of the breast , prostate , stomach and melanoma cancers but overexpressed in pancreatic , gall bladder , colorectal ,skin and thyroid cancers.

This varying function is because of its varying subcellular localization and its interaction with extacellular matrix and its epigenetic modification(70,71,72,73).

Maspin G - alpha helix an internal salt bridge or the p1 position of the RCL.

It is capable of an open and closed conformational change inducing redistribution of charged residues within the molecule .

Effect of maspin on cell migration depends on G –alpha helix and its action on alpha 1 integrins[80]. Maspin induces the changes in the expression of protein associated with actin cytoskeleton that predicts a less motile and invasive phenotype and reduced metastatic spread[81,82] . Action of maspin on cell migration is also the result of inactivation of beta 1 integrins sub unit.

Tumour metastasis requires the cell detachment and invasion through the basement membrane and stroma. During metastasis of tumour many genes are reduced or silenced during this process.

Maspin is also silenced during this process and promotes cell invasion , angiogenesis and more recently apoptosis. Maspins role on protease inhibitor is considered to be supressing activity in tumour metastasis.

Maspin is epigenetically regulated in tissue specific way. Epigenetic changes of maspin expression involves cytosine methylation, histone deacetylation and chromatin accessibility causing loss of its function .[87,88,89]

ROLE OF MASPIN IN HEAD AND NECK CANCER :

Maspin represents an useful marker to identify the potential for progression of head and neck cancer , since lower immunoreactivity is associated with larger tumour and greater invasive potential .

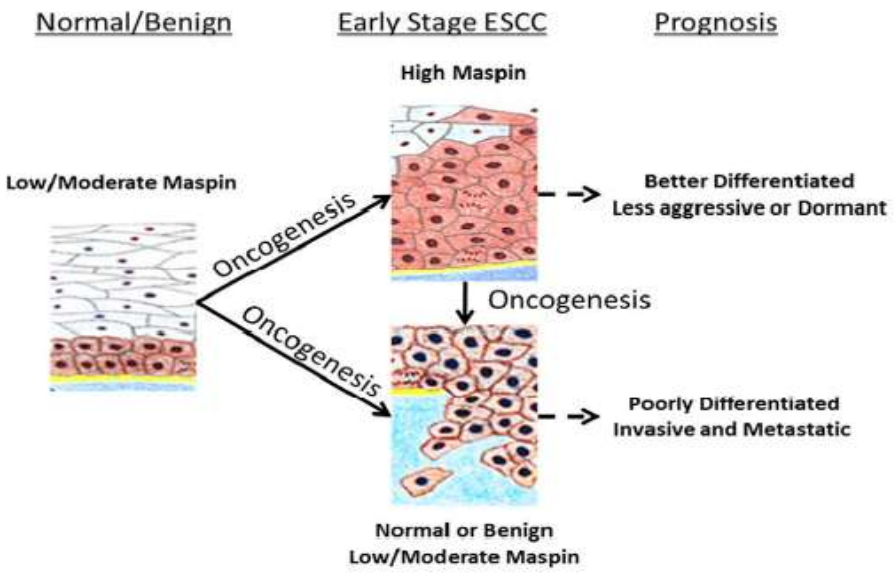


Fig 4 : Hypothetical Model Of Maspin Expression In Oral Squamous Cell Carcinomas

Future perspectives : Maspin expression was found directly correlated with treatment including carboplatin with radiotherapy in primary head and neck squamous cell carcinoma.[90]

Thus maspin shows a positive prognostic value. Therapeutic approaches studied so far aimed to reactivate a dormant tumour supressor gene by designed transcription factors[91].

MATERIALS AND METHODS

STUDY DESIGN : Retrospective and comparative study

SOURCE OF DATA : Incisional biopsy / Excision biopsy specimen of oral cavity lesion was obtained from Department of Oto Rhinolaryngology and Surgery and reported by Department Of Pathology ,Govt Stanely Medical College ,Chennai.

STUDY PERIOD : This study was from January 2016 to December 2016

PILOT STUDY : Done with 5 specimens in September 2015 submitted to institutional ethical committe in October 2016 and approval obtained .

SAMPLE SIZE ESTIMATION : Level of expression of maspin and intensity of staining in malignant cells was considered as the primary variable in calculating the sample size .

TOTAL SAMPLE SIZE : 88

STUDY GROUPS :

Group 1 : Specimen diagnosed as malignant by Histopatholgical Examinantion – Well Differentiated SCC

Group 2 : Specimen diagnosed as malignant by Histopatholgical Examinantion – Moderately Differentiated

Group 3 : Specimen diagnosed as malignant by Histopatholgical Examinantion – Poorly Differentiated

RANDOM SELECTION :

All cases were selected by simple randomisation . Out of all specimens reported during January 2016to December 2016; 36 were Well Differentiated Squamous Cell Carcinoma 36 were Moderately Differentiated Squamous Cell Carcinoma; 16 were Poorly Differentiated Squamous Cell Carcinoma.

INCLUSION CRITERIA:

All Histopathological variants of Oral Squamous Cell Carcinoma reported in Department Of Pathology ,Stanley Medical College during the period of January 2016 to December 2016 sent from Department Of Surgery and Otorhinolaryngology.

EXCLUSION CRITERIA :

1) Benign neoplasm arising from the oral mucosa will not be included in this study.

2) Primary malignancies of oral cavity other than Squamous Cell Carcinoma will not be included in this study.

3) Recurrence and Metastatic tumours of the oral cavity.

METHODS OF STUDY :

Detailed history regarding the patients age , gender ,site , personal history were collected from the surgical pathology records.

Sections of 4 micrometer thickness were taken from the corresponding paraffin blocks by using semi automated microtome with disposable blades and followed by staining with Haematoxylin and Eosin stain. The stained sections were then reviewed.

Sections showing normal histology ,features of dysplasia and features of carcinoma were named accordingly. Malignant cases were further categorized into three grades. With or without nodal metastasis was further categorized.

36 cases of well differentiated , 36 cases of moderately differentiated , 16 cases of poorly differentiated with or without nodal metastasis were selected at random for staining with MASPIN immunohistochemical marker. The corresponding paraffin blocks of the above selected cases were taken. Sections were cut at 4 micrometer thickness in semiautomated microtome using disposable blades. Chrome alum slides were used for this purpose.

Sections were subjected to antigen retrival solution using pressure cooker technique with TRIS buffer solution corresponding to PH 9.0 and was subjected to bind with mouse monoclonal antibody (Thermofisher) against MASPIN. This was further proceeded with seconday antibody and finally diaminobenzidine substrate . The entire steps of this procedure is explained below.

IMMUNOHISTOCHEMISTRY PROCEDURE

1. 4µ thick sections were cut from formalin fixed paraffin embedded tissue samples and transferred to gelatin-chrome alum coated slides.

2. The slides were incubated at 58ºC for overnight.

3. The sections were deparaffinized in xylene for 15 minutes x 2 changes.

4. The sections were dehydrated with absolute alcohol for 5 minutes x 2 changes.

5. The sections were washed in tap water for 10 minutes.

6. The slides were then immersed in distilled water for 5 minutes.

7. Heat induced antigen retrieval was done with microwave oven in appropriate temperature with appropriate buffer for 20 to 25 minutes.

8. The slides were then cooled to room temperature and washed in running tap water for 5 minutes.

9. The slides were then rinsed in distilled water for 5 minutes.

10. Wash with appropriate wash buffer (citrate buffer) for 5 minutes x 2 changes.

11. Apply peroxidase block over the sections for 10 minutes.

12. Wash the slides in citrate buffer for 5 minutes x 2 changes.

13. Cover the sections with power block for 15 minutes.

14. The sections were drained (without washing) and appropriate primary antibody MASPIN was applied over the sections and incubated for 30 minutes .

15. The slides were washed in citrate buffer for 5 minutes x 2 changes.

16. The slides were covered with Super Enhancer for 30 minutes.

17. The slides were washed in citrate buffer for 5 minutes x 2 changes.

18. The slides were covered with SS Label for 30 minutes.

19. Wash in citrate buffer for 5 minutes x 2 changes.

20. DAB substrate was prepared by diluting 1 drop of DAB chromogen to 1 ml of DAB buffer.

21. DAB substrate solution was applied on the sections for 8 minutes.

22. Wash with citrate buffer solution for 5 minutes x 2 changes.

23. The slides are washed well in running tap water for 5 minutes.

24. The sections were counterstained with Hematoxylin stain for 2 seconds (1 dip).

25. The slides were washed in running tap water for 3 minutes.

26. The slides were air dried, cleared with xylene and mounted with DPX..

ANTIGEN	VENDOR	SPECIES	DILUTION	POSITIVE CONTROL
MASPIN	THERMOFISHER	MOUSE	READY TO USE(NO DILUTION)	Normal oral squamous epithelium

INTERPRETATION :

Immunohistochemical slides were labelled , viewed and analysed . The immunopostivity was confirmed by the presence of brown coloured staining in the cytoplasm of the tumour cells.

Evaluation of expression of immunohistochemical expression of Maspin in tumour cells :

Maspin expression was semi quantitatively evaluated by percentage of cells positive for maspin staining and the intensity of maspin staining .[(93)].

- Percentage of positive cells was scored as follows:
  - ❖ 0 point- Maspin positive in 0–5% of cells
  - ❖ 1 point – Maspin positivity in 5-10% of cells
  - ❖ 2 points -Maspin positive in 6–50% of cells
  - ❖ 3 points- Maspin positive in more than 50% of cells
- The staining intensity was scored as follows:
  - ❖ 1 point: negative or weak staining
  - ❖ 2 points: moderate staining
  - ❖ 3 points: strong staining.

The **OVERALL MASPIN EXPRESSION (OME)** was assessed based on the percentage of positive cells category points and the intensity category points in each case.

Tumors were categorized into four groups:

- ❖ NEGATIVE : < 5% of cells stained, regardless of intensity
- ❖ WEAK EXPRESSION : OME: 0–2 points
- ❖ MODERATE EXPRESSION : OME: 3–4 points

- ❖ STRONG EXPRESSION : OME: 5–6 points

PARAMETERS STUDIED :

The following parametes were evaluated :

1. Age
2. Gender
3. Site
4. Histopathology report
5. Overall Maspin Expression (OME)

   Results were tabulated accordingly and analysed.

STATISTICAL ANALYSIS

Totally 9374 cases were reported in the Govt Stanley Medical College during the period of January 2016 to December 2016 . 246 cases were taken from the oral cavity . Out of which 153 cases were reported as malignant. Among malignant cases 88 cases were randomly selected .

OVERALL MASPIN EXPRESSION (OME) is considered as the primary outcome variable. Various histological grades of tumours and nodal status are considered as the explanatory variables. The sociodemographic variables like age , gender, were considered as other explanatory variables

Descriptive analysis: Descriptive analysis was carried out by mean and standard deviation for quantitative variables, frequency, and proportion for categorical variables. Data was also represented using appropriate diagrams like bar diagram, pie diagram, and box plots.

INFERENTIAL STATISTICS:

Quantitative outcome;

The association between categorical explanatory variables and the quantitative outcome was assessed by comparing the mean values. The mean differences along with their 95% CI were presented. Independent sample t-test was used to assess statistical significance.

Categorical outcome:

The association between explanatory variables and categorical outcomes was assessed by cross tabulation and comparison of percentages. Chi square test was used to test statistical significance.

P value < 0.05 was considered statistically significant. IBM SPSS version 22 was used for statistical analysis

OBSERVATION AND RESULTS:

A total of 88 subjects were included in the analysis.

Table 1: Descriptive analysis for AGE in study population (N=88)

Parameter	Mean ± STD	Median	Min	Max	95% C.I. for EXP(B)	
					Lower	Upper
AGE	53.55 ± 10.09	55.00	32.00	75.00	51.42	55.70

The mean age of study population was 53.55 with minimum age being 32 years and maximum being 75 years in this study population (95% CI 51.42- 55.70).

---**Figure 1: Box and whisker plots of AGE in the study population(N=88)**

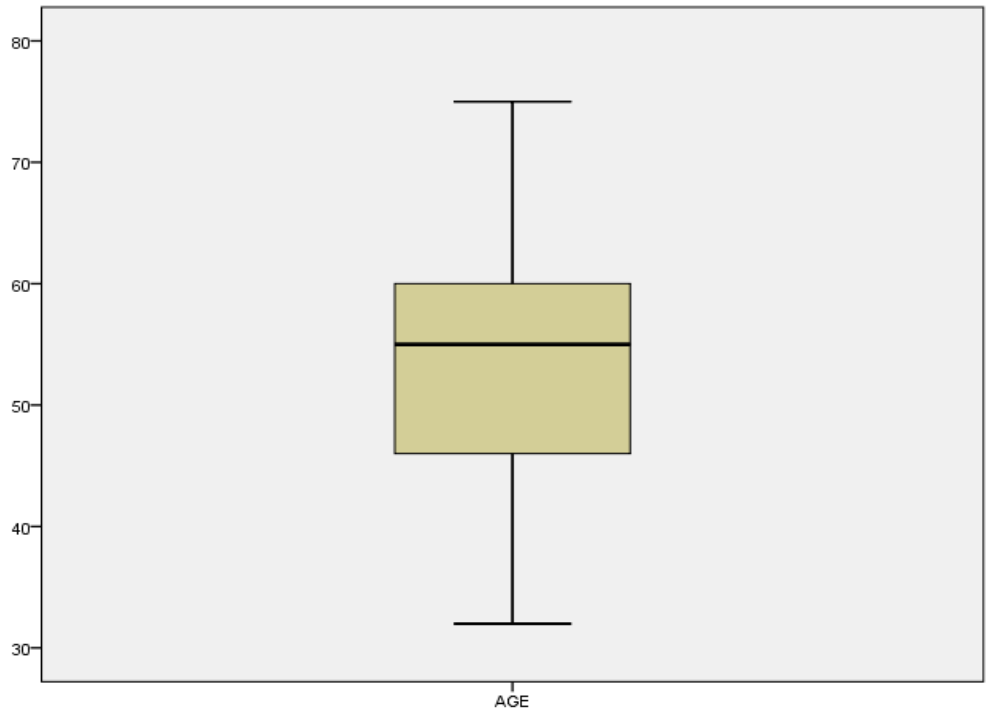


Table 2: Descriptive analysis of GENDER in study population (N=88)

GENDER	Frequency	Percentage
MALE	73	82.95%
FEMALE	15	17.05%

Among the study population, number of males 73(82.95%) was higher than females 15(17.05%). (table 2)

Figure 2: Bar chart of GENDER distribution in study population (N=88)

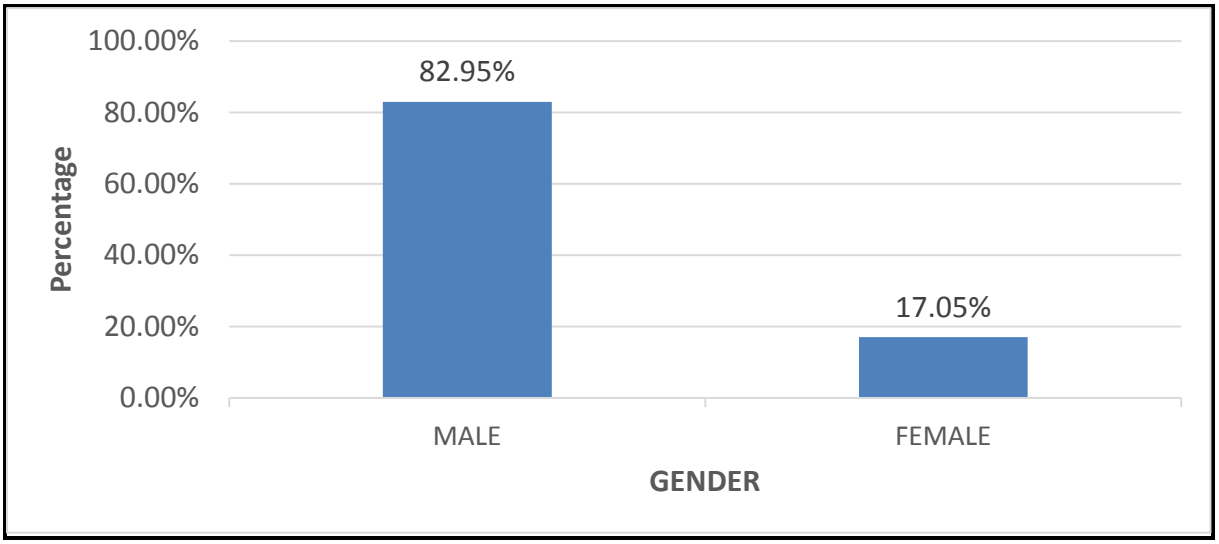


Table 3: Descriptive analysis of SITE in study population (N=88)

Parameter	Frequency	Percentage
BUCCAL MUCOSA	21	23.86%
VOCAL CORD	10	11.4%
TONGUE ANTERIOR 2/3	19	21.59%
HYPO PHARYNX	9	10.23%
TONGUE POSTERIOR 1/3	9	10.23%
LIP	6	6.82%
RETROMOLAR TRIGONE	4	4.55%
OROPHARYNX	3	3.41%
SUPRAGLOTTIS	2	2.3%
GLOTTIS	2	2.3%
HARD PLATE	1	1.14%
PYRIFORM FOSSA	1	1.13%
EPIGLOTTIS	1	1.13%

Among the study population, the most common site of involvement was buccal mucosa in 21 (23.86%) subjects. It was followed by anterior two third of tongue 19(21.59%), vocal cord in 10(11.4%) , hypo pharynx and posterior one third tongue in 9 (10.23%) subjects each. The summary of other sites involved is presented in table 3. (Table 3)

Figure 3: Bar chart of SITE distribution in study population (N=88)

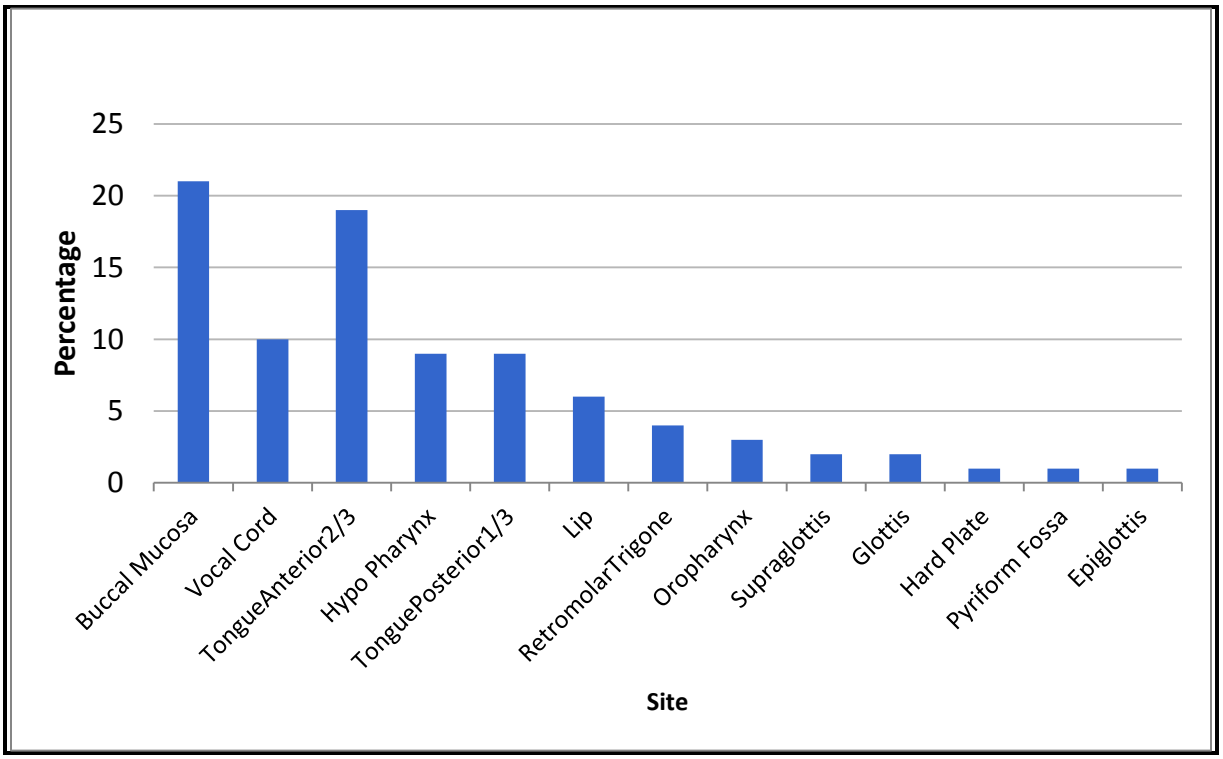


Table 4: Descriptive analysis of Tumour stage and Nodal status in study population (N=88)

Parameter	Frequency	Percentages
I.T		
T1	43	48.86%
T2	39	44.32%
T3	6	6.82%
II.N		
N0	56	63.64%
N1	15	17.05%
N1 A	3	3.41%
N1 b	1	1.14%
N2	3	3.41%
N2 a	2	2.27%
N2 b	7	7.95%
N3	1	1.13%

Among the study population, as per the TNM staging, majority of the study population were in T1 (48.86%). The number of subjects in T2 and T3 were 39(44.32%), and 6(6.82%) respectively. The proportion of subjects with no nodal involvement was 63.64% in study population. The proportion of subjects in N1, N1 A and N1b categories were 17.05%, 3.41% and 1.14% respectively. The proportion of subjects in N2, N2a and N2b categories were 3.14%, 2.27% and 7.95% respectively. Only 1 (1.135) subject was in N3 category. (Table 4)

Table 5: Descriptive analysis of nodal metastasis in study population (N=88)

N	Frequency	Percentages
Nodal metastasis present	32	36.36%
Nodal metastasis absent	56	63.64%

Among the study population, the proportion of subjects with any nodal metastasis was 36.36%. (Table 5)

Fig4: Pie chart of N distribution in study group (N=88)

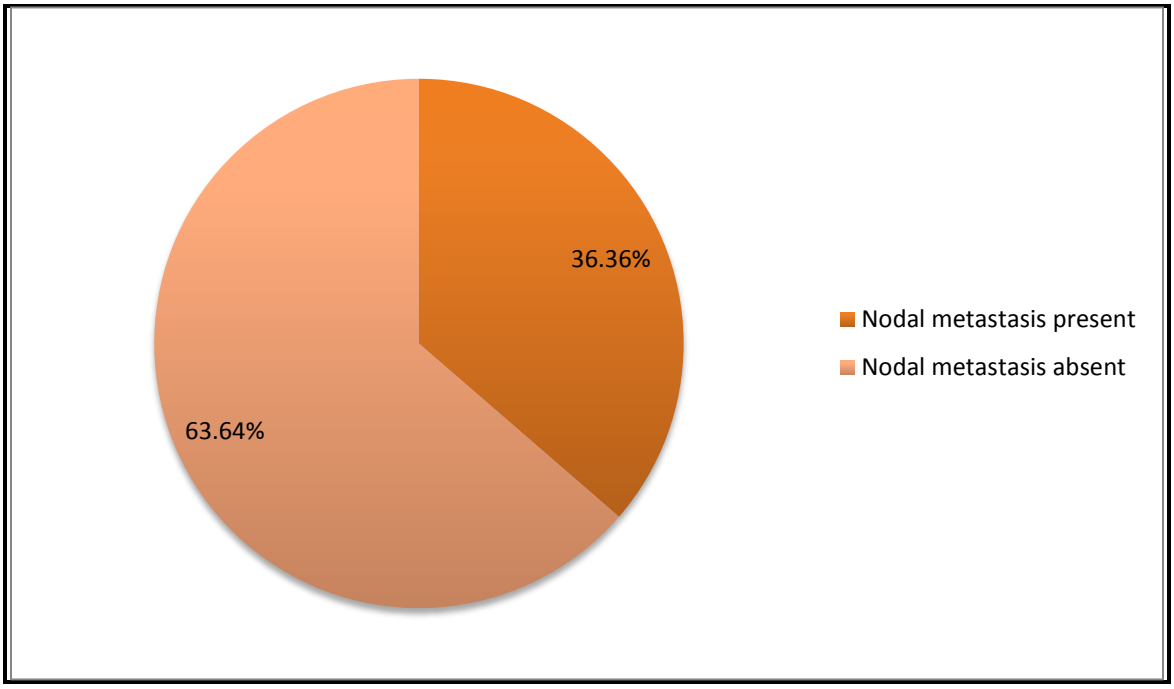


Table 6: Descriptive analysis of Grade in study population (N=88)

Grade	Frequency	Percentages
I	36	40.91%
II	36	40.91%
III	16	18.18%

Among the study population, the tumor was Grade I in 36 (40.91%), Grade II in 36 (40.91%) and Grade III in 16(18.18%) subjects. (Table 6)

Figure 5: Pie chart of Grade distribution in study population (N=88)

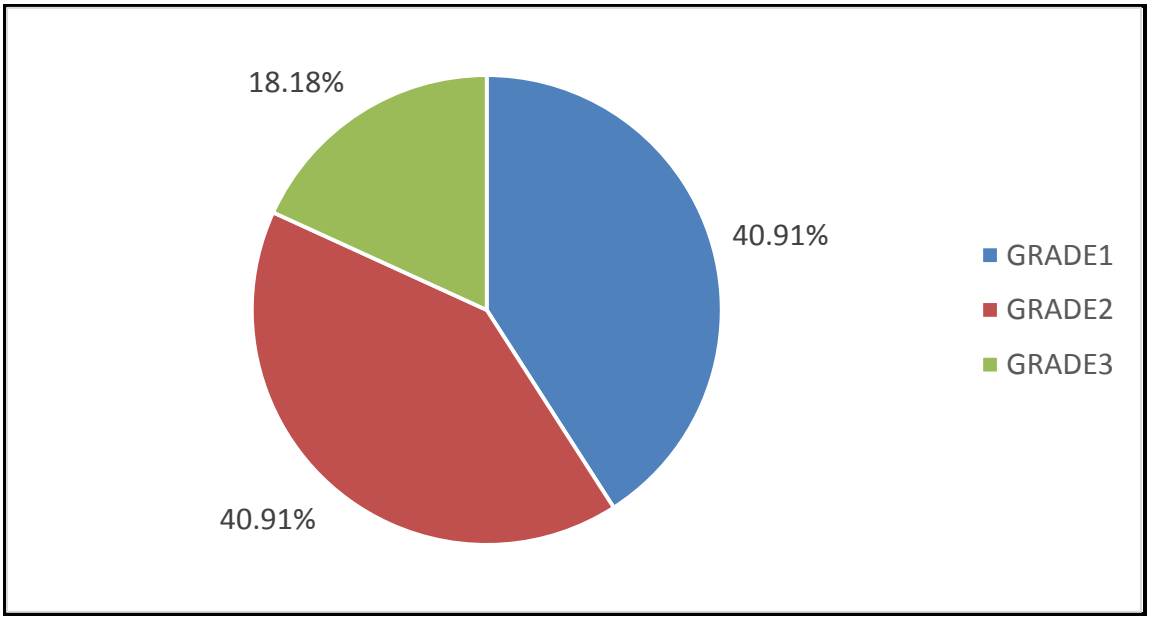


Table7 : Descriptive analysis of Stage in study population (N=88)

STAGE	Frequency	Percentages
I	32	36.36%
II	22	25.00%
III	21	23.86%
IVA	12	13.64%
IV B	1	1.14%

Among the study population. The staging of tumor was Stage I in 32(36.36%), Stage II in 22(25.00%), Stage III in 21(23.08%) people. Stage IVA and Stage IV B tumor was present in 12(13.64%)and 1(1.14%) subject respectively. (Table 7)

Fig6: Pie chart of STAGE distribution in study group (N=88)

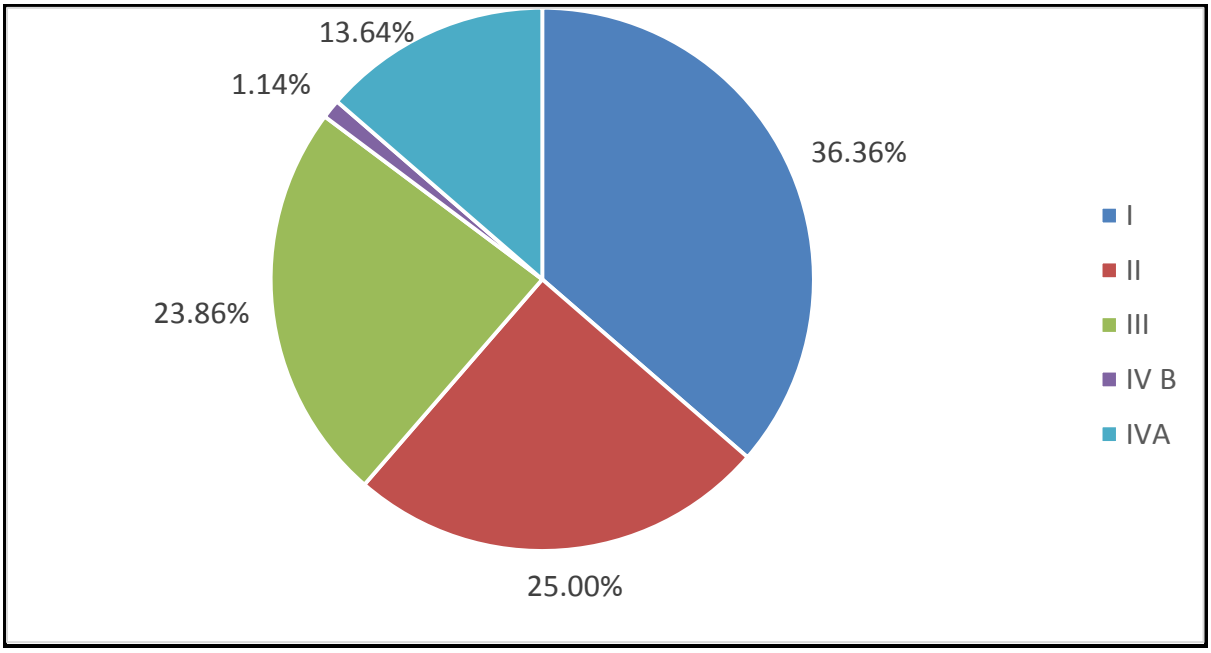


Table8: Descriptive analysis of intensity of Expression in study population (N=88)

Intensity of Expression	Frequency	Percentage
Negative	28	31.82%
Weak	13	14.77%
Moderate	24	27.27%
Strong	23	26.14%

Among the study populationIntensity of Expression was Negativein 28(31.82%) people. The intensity expression was Weak in 13(14.77%), Moderate in 24(27.27%) and Strong in 23(26.14%) subjects. (Table 8)

Figure 7: Bar chart of intensity of Expression distribution in study population (N=88)

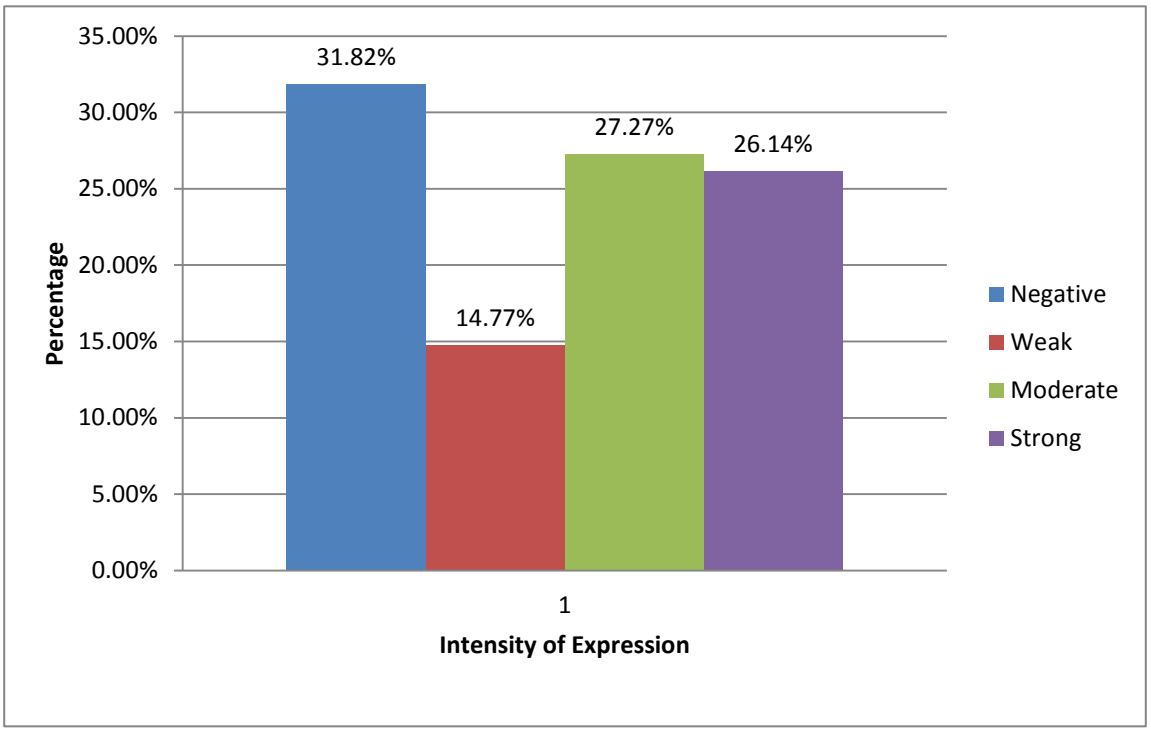


Table9: Descriptive analysis for LENGTH, WIDTH, HEIGHT, TOTAL AREA in study population (N=88)

Parameter	Mean±STD	Median	Min	Max	95% C.I. for EXP(B)	
					Lower	Upper
LENGTH	3.066±1.401	3.50	1.50	4.20	-0.41	6.55
WIDTH	1.833±1.154	2.50	0.50	2.50	-1.04	4.70
HEIGHT	1.166±0.577	1.50	0.50	1.50	-0.27	2.60
TOTAL AREA	8.291±7.697	8.75	0.38	15.75	-10.83	27.41

The Mean total area of study population was 8.291 with minimum 0.38 and maximum 15.75 area in the study population(95%CI-10.83-27.41).

Table10: Descriptive analysis of score for NUMBER OF CELLS STAINED AND INTENSITY OF STAINING in study population(N=88)

Parameter	Frequency	Percent
I.NUMBER OF CELLS STAINED		
0	29	32.95%
1	12	13.64%
2	26	29.55%
3	21	23.86%
II.INTENSITY OF STAINING		
0	12	13.64%
1	31	35.23%
2	30	34.09%
3	15	17.05%

Among the study population, number of cells stained was scored as 0,1,2and 3 according to the numbe of cells stained . The score was 0,1,2 and 3 respectively in 29(32.95%),12(13.64%), 26(29.55%) and 21(23.865)cases .The intensity of staining was also graded 0,1,2 and 3 which is 12(13.64%), 31(35.23%), 30(34.09%), and 15 (17.05%) subjects respectively. (Table 10)

Table11: Association of OVERALL MASPIN EXPRESSION (OME) with GRADE of study population (N=88)

GRADE	OVERALL MASPIN EXPRESSION (OME)				Chi square	P-value
	Negative	Weak	Moderate	Strong		
I	7 (19.44%)	5 (13.88%)	6 (16.66%)	18 (50%)		
II	10 (27.77%)	5 (13.88%)	16 (44.44%)	5 (13.88%)	29.73	<0.001
III	11 (68.75%)	3 (18.75%)	2 (12.5%)	0 (0%)		

Among grade I subjects, the proportion of subjects with Overall Maspin Expression (OME) Expression Negative was 7 (19.44%), Weak was 5 (13.88%), Moderate was 6 (16.66%) and strong was 18 (50%).The proportion of subjects with OVERALL MASPIN EXPRESSION (OME) Expression Negative was 10 (27.77%), Weak was 5 (13.88%), Moderate was 16 (44.44%) and strong was 5 (13.88%)in subjects with Grade II. The proportion of subjects with Overall Maspin Expression (OME) Expression Negative was 11 (68.75%), Weak was 3 (18.75%), Moderate was 2 (12.5%)

and strong was 0 (0%) in subjects with GradeIII.The differences in proportion Across the groups was statistically significant (P value<0.001). (Table 11).

Fig 8: Bar chart of OVERALL MASPIN EXPRESSION (OME) Expression between Grade distribution in study group (N=88)

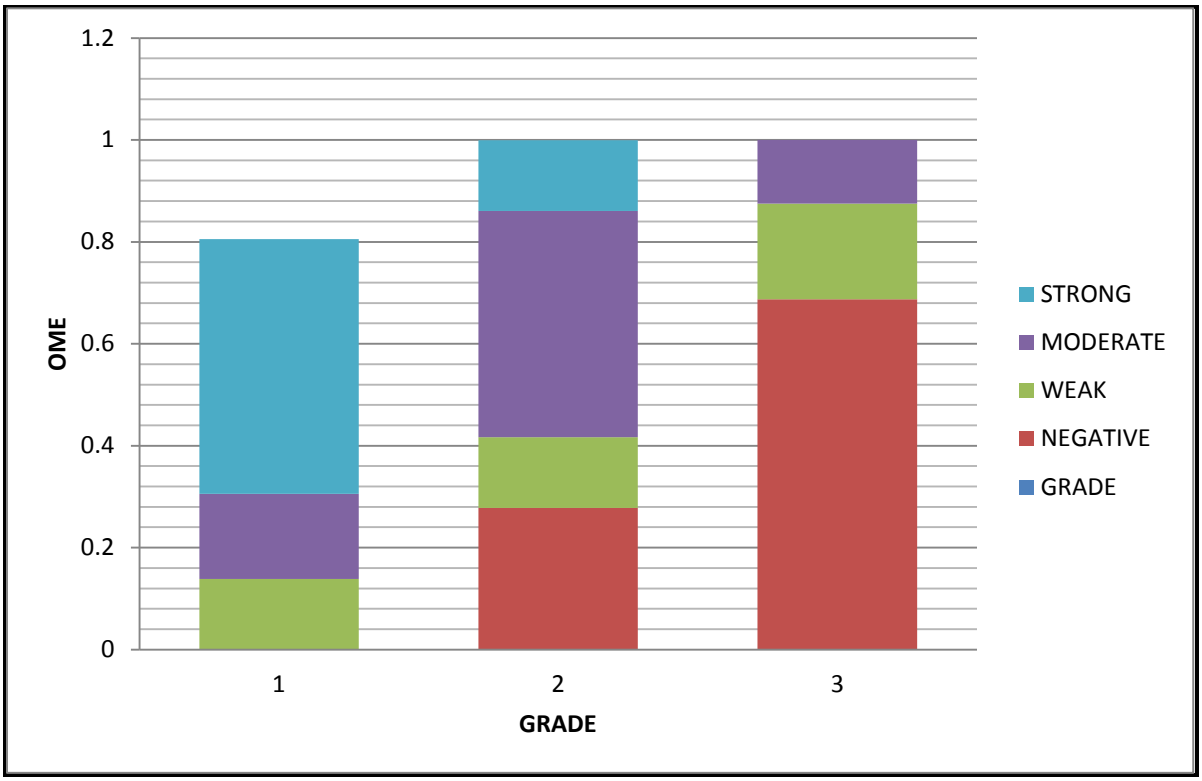


Table 12: Association of OVERALL MASPIN EXPRESSION (OME) Expression with N of study population (N=88)

N	OVERALL MASPIN EXPRESSION (OME)				Chi square	P-value
	Negative	Weak	Moderate	Strong		
Nodal metastasis present	24 (75%)	7(21.87%)	1 (3.125%)	0 (0%)	55.08	<0.001
Nodal metastasis Absent	4 (7.142%)	6(10.71%)	23 (41.07%)	23(41.07%)		

Among people with nodal metastasis, the proportion of subjects with OVERALL MASPIN EXPRESSION (OME) Negative was 24 (75%), Weak was 7 (21.87%), Moderate was 1 (3.125%) and Strong was 0 (0%). In people without nodal metastasis, the proportion of subjects with Overall Maspin Expression (OME) Negative was 4 (7.142%), Weak was6(10.71%), Moderate was 23 (41.07%)and Strong was 23(41.07%)The differences in proportion between the two groups was statistically significant (P value<0.001). (Table 13).

Fig 9: Bar chart of OVERALL MASPIN EXPRESSION (OME) between N distribution in study group (N=88)

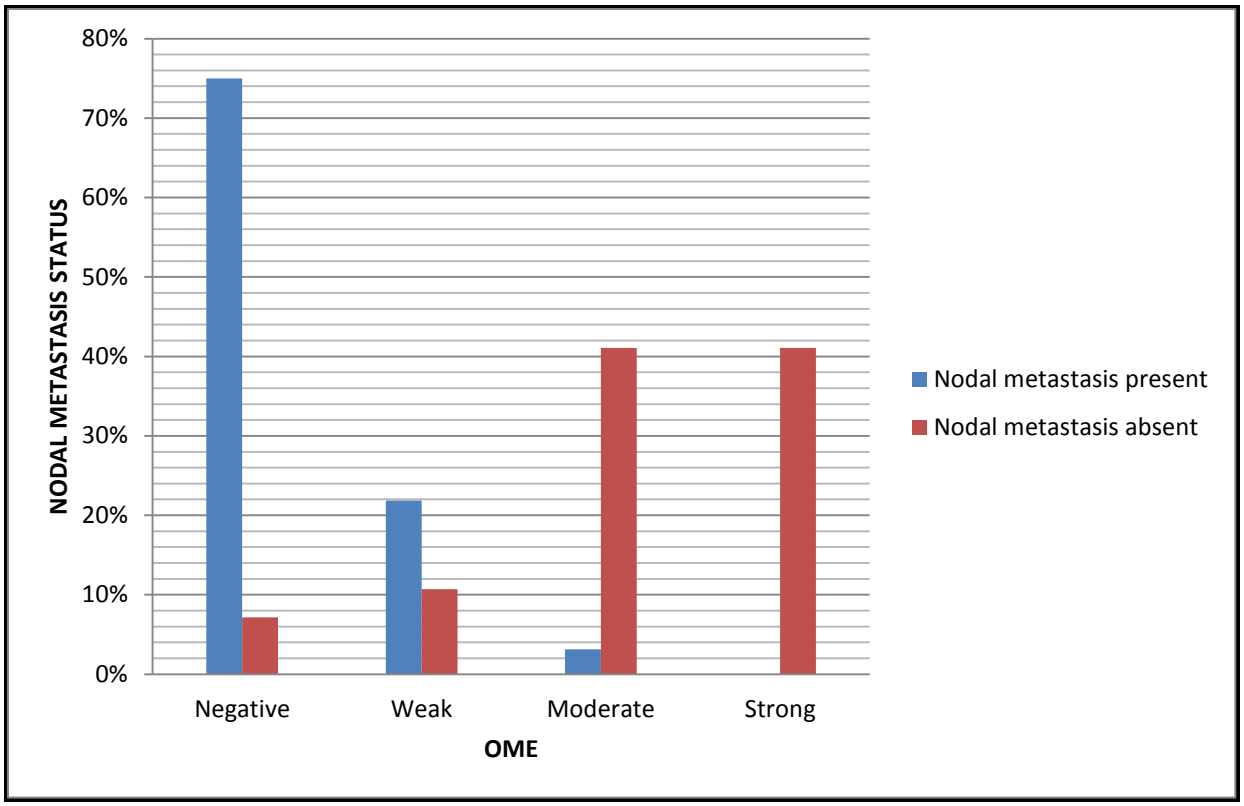


Table 13: Association of OVERALL MASPIN EXPRESSION (OME) with STAGE of study population (N=88)

STAGE	OVERALL MASPIN EXPRESSION (OME)				Chi square	P-value
	Negative	Weak	Moderate	Strong		
I	0 (0%)	3(9.375%)	11 (34.37%)	18(56.25%)	68.07	*<0.001*
II	3 (13.63%)	3(13.63%)	11 (50%)	5 (22.72%)		
III	13 (61.90%)	6(28.57%)	2 (9.523%)	0 (0%)		
IV B	1 (100%)	0 (0%)	0 (0%)	0 (0%)		
IVA	11 (91.66%)	1(8.333%)	0 (0%)	0 (0%)		

Among Stage I subjects, the proportion of subjects with OVERALL MASPIN Expression was Negative in 0 cases (0%), Weak in 3(9.375%), Moderate in 11 (34.37%) and was strong in 18(56. 25%).The proportion of subjects with Expression Negative was 3 (13.63%), Weak was 3(13.63%),

Moderate expression was seen in 11 (50%)and strong expression was seen in 5 (22.72%) subjects with Stage II. The proportion of subjects with Overall Maspin Expression was negative in 13 (61.90%), Weak in 6(28.57%), Moderate in 2 (9.523%) and strong in 0 (0%) of subjects with Stage III. The proportion of subjects with Overall Maspin Expression was negative in 1 (100%), Weak in 0 (0%), Moderate in 0 (0%) and

strong in 0 (0%) of subjects with Stage IVB.The proportion of subjects with Expression was negative in 11 (91.66%)Weak in 1(8.333%), Moderate in 0 (0%) and strong in 0 (0%) subjects with Stage IVA.The differences in proportion across the groups was statistically significant (P value<0.001). (Table 14).

Fig 10: Bar chart of OVERALL MASPIN EXPRESSION (OME) between Stage distribution in study group (N=88)

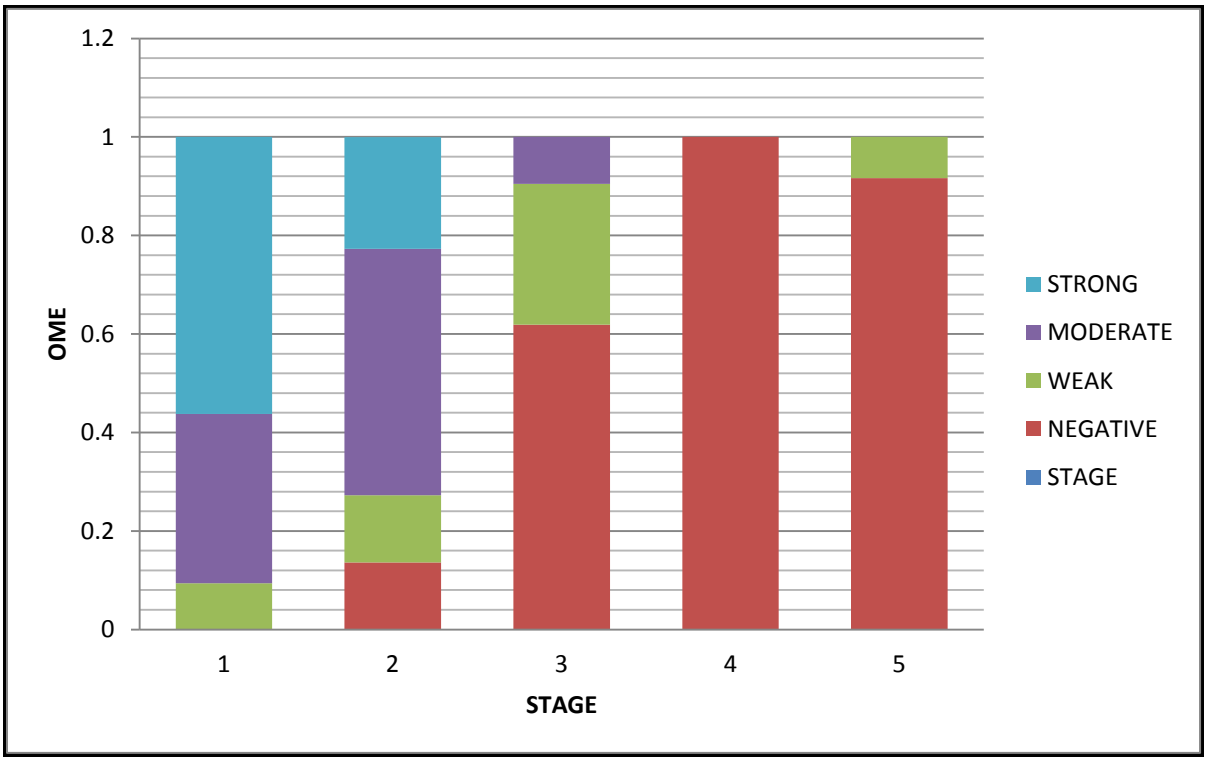


Table 14: Association of INTENSITY OF STAINING with GRADE of study population (N=88)

GRADE	INTENSITY OF STAINING				Chi square	P - value
	0	1	2	3		
I	3 (8.333%)	8(22.22%)	15(41.66%)	10(27.77%)	26.107	<0.001
II	2 (5.555%)	15(41.66%)	14(38.88%)	5 (13.88%)		
III	7 (43.75%)	8 (50%)	1 (6.25%)	0 (0%)		

Among grade I subjects, the proportion of subjects with intensity of staining 0 was 3 (3 (8.333%), 1 was 8 (22.22%), 2 was 15 (41.66%) and 3 was 10 (27. 77%).The proportion of subjects with intensity of staining 0 was 2 (5.555%), 1 was 15(41.66%),2 was 14(38.88%)and 3 was 5 (13.88%)in subjects with Grade II. The proportion of subjects with intensity of staining0 was7 (43.75%), 1 was 8 (50%), 2 was 1 (6.25%)and 3 was 0 (0%)in subjects with GradeIII.The differences in proportion Across the groups was statistically significant (P value<0.001). (Table 15).

Fig 11: Bar chart of INTENSITY OF STAINING between grade distribution in study group (N=88)

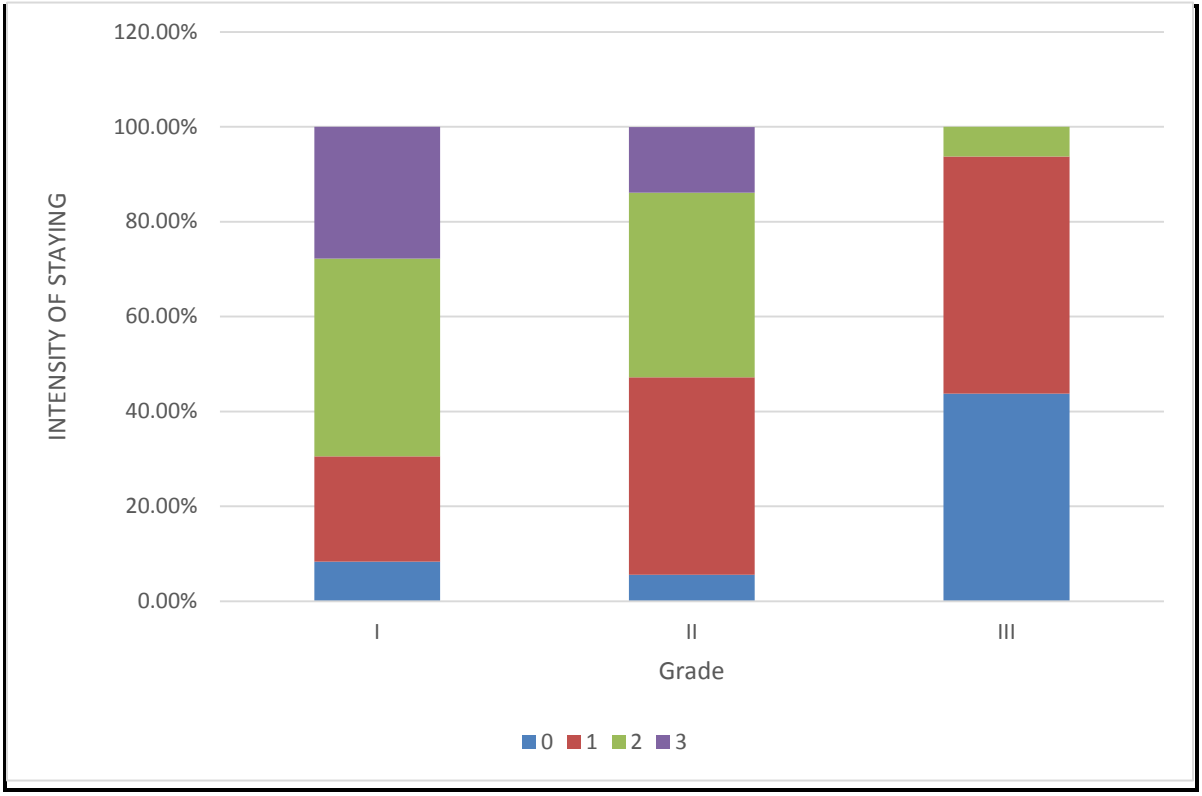


Table 15: Association of INTENSITY OF STAINING with N of study population (N=88)

N	INTENSITY OF STAINING				Chi square	P-value
	0	1	2	3		
Nodal metastasis present	10 (31.25%)	17(53.12%)	4 (12.5%)	1(3.125%)	28.60	*<0.001*
Nodal metastasis absent	2 (3.571%)	14 (25%)	26(46.42%)	14 (25%)		

Among people with nodal metastasis, the proportion of subjects with intensity of staining 0 was 10 (31.25%), 1 was 17(53.12%),2 was 4 (12.5%)and 3 was 1(3.125%). In people without nodal metastasis, the proportion of subjects with intensity of staining 0was 2 (3.571%), 1 was 14 (25%), 2 was 26(46.42%)and 3was 14 (25%).The differences in proportion between the two groups was statistically significant (P value<0.001).(Table 16).

Fig 12: Bar chart of INTENSITY OF STAINING between N distribution in study group (N=88)

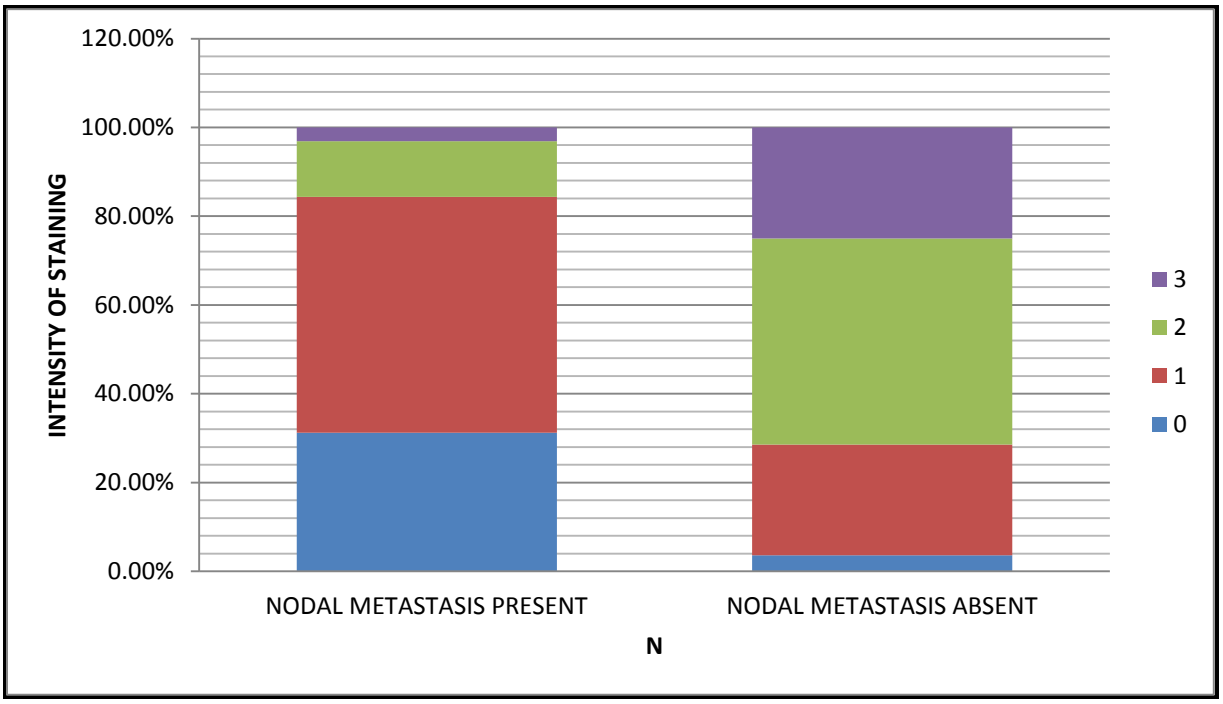


Table 16: Association of INTENSITY OF STAINING with STAGE of study population (N=88)

STAGE	INTENSITYOF STAINING				Chi square	P-value
	0	1	2	3		
I	0 (0%)	9 (28.12%)	12 (37.5%)	11(34.37%)	61.30	<0.001
II	1 (4.545%)	5 (22.72%)	13(59.09%)	3 (13.63%)		
III	3 (14.28%)	13(61.90%)	5 (23.80%)	0 (0%)		
IVA	8 (66.66%)	4 (33.33%)	0 (0%)	0 (0%)		
IV B	0 (0%)	0 (0%)	0 (0%)	1 (100%)		

Among Stage I subjects, the proportion of subjects with intensity of staining 0 was 0 (0%), 1 was 9 (28.12%), 2 was 12 (37.5%)and 3 was 11(34.37%). The proportion of subjects with intensity of staining 0 was 1 (4.545%), 1 was 5 (22.72%), 2 was 13(59.09%)and 3 was 3 (13.63%)in subjects with Stage II. The proportion of subjects with intensity of staining 0 was 3 (14.28%), 1 was 13(61.90%), 2 was 5 (23.80%) and 3 was 0 (0%) in subjects with Stage III. The proportion of subjects with intensity of staining 0 was 8 (66.66%), 1 was 4 (33.33%), 2 was 0 (0%) and 3 was 0 (0%) in subjects with Stage IVA.The proportion of subjects with intensity of staining 0 was 0 (0%)1was 0 (0%) ,2 was 0 (0%) and3was 1 (100%)in subjects with Stage IVB. The differences in proportion across the groups was statistically significant (P value<0.001). (Table 16).

COLOUR PLATES

FIGURE 5: SQUAMOUS CELL CARCINOMA– LATERAL BORDER OF TONGUE

FIGURE 6 : SQUAMOUS CELL CARCINOMA – GLOTTIS

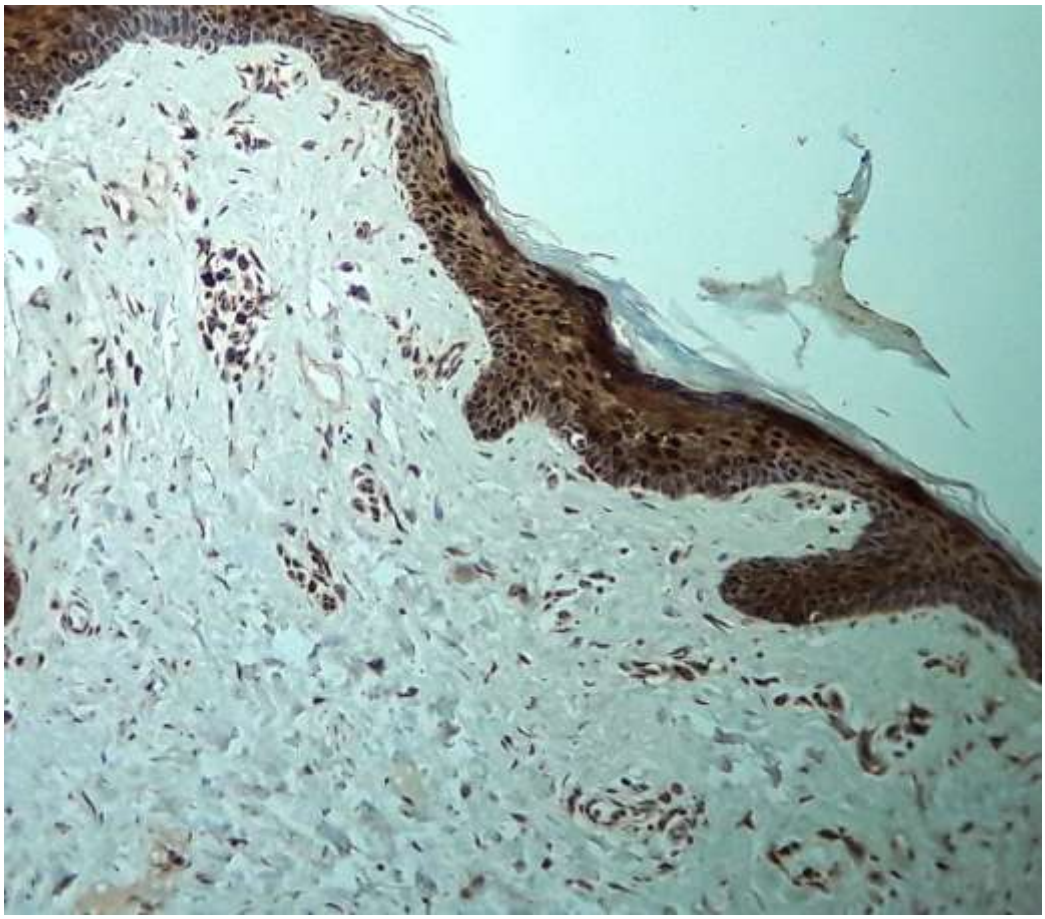


FIGURE 7 : CONTROL – NORMAL SQUAMOUS EPITHELIUM

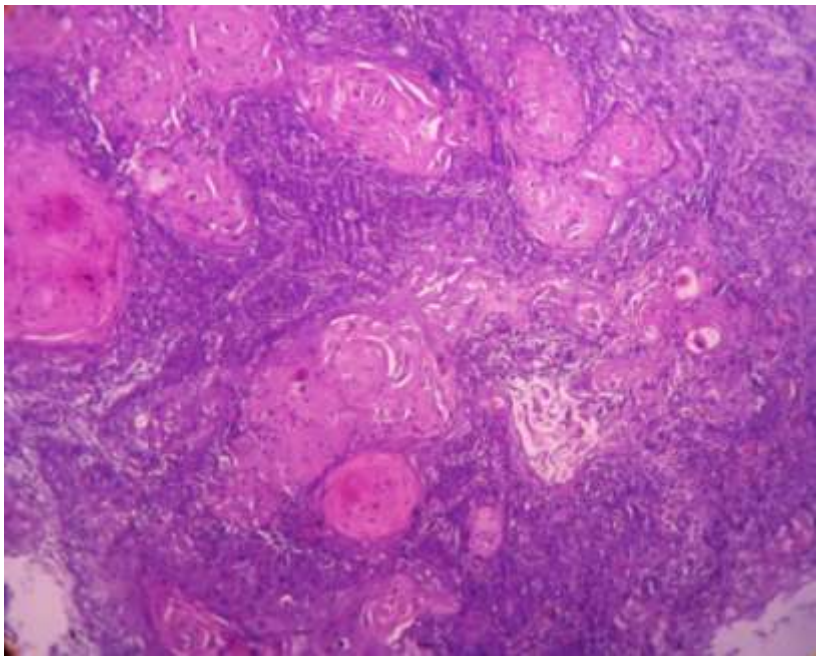


FIGURE 8 :WELL DIFFERENTIATED SQUAMOUS CELL CARCINOMA

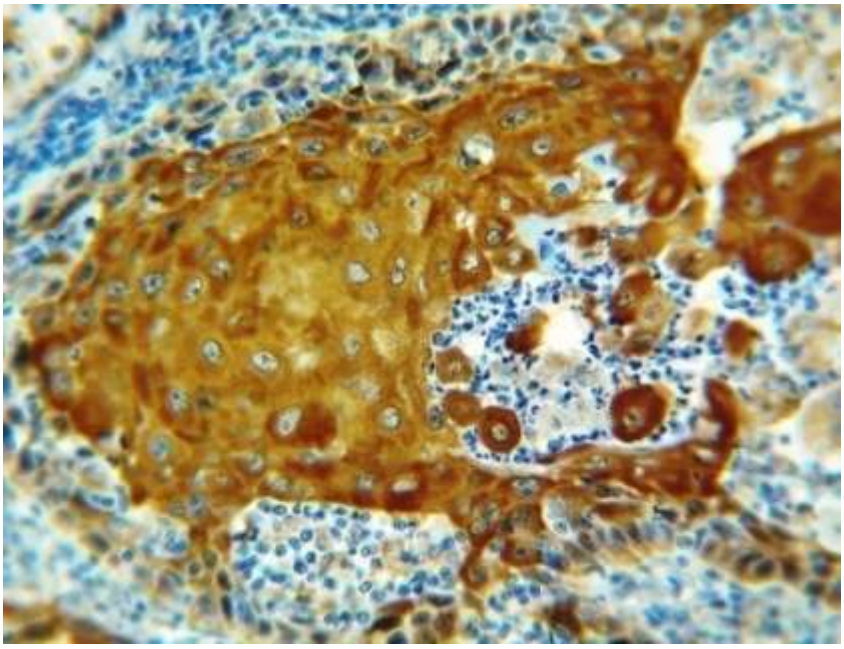


FIGURE 9 :WELL DIFFERENTIATED SQUAMOUS CELL CARCINOMA SHOWS STRONG POSITIVITY OF MASPIN

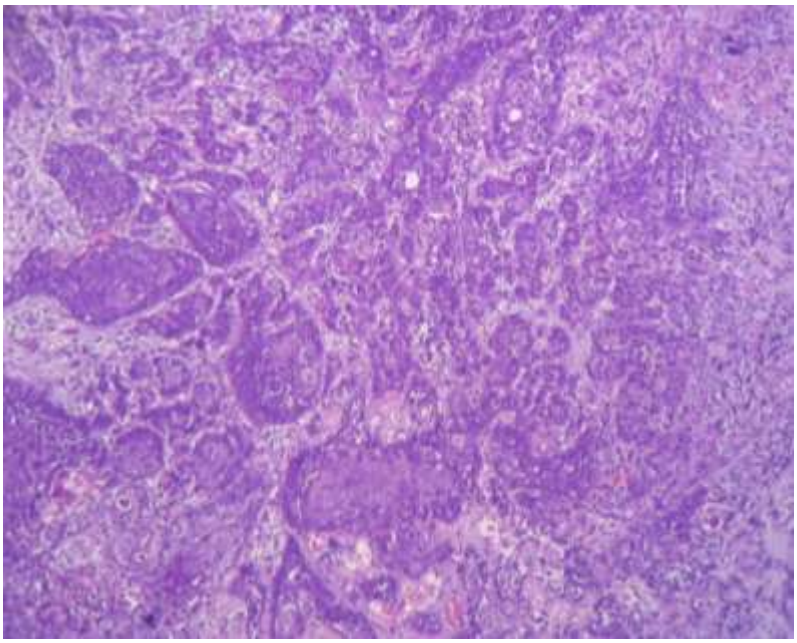


FIGURE 10 : MODERATELY DIFFERENTIATED SQUAMOUS CELL CARCINOMA

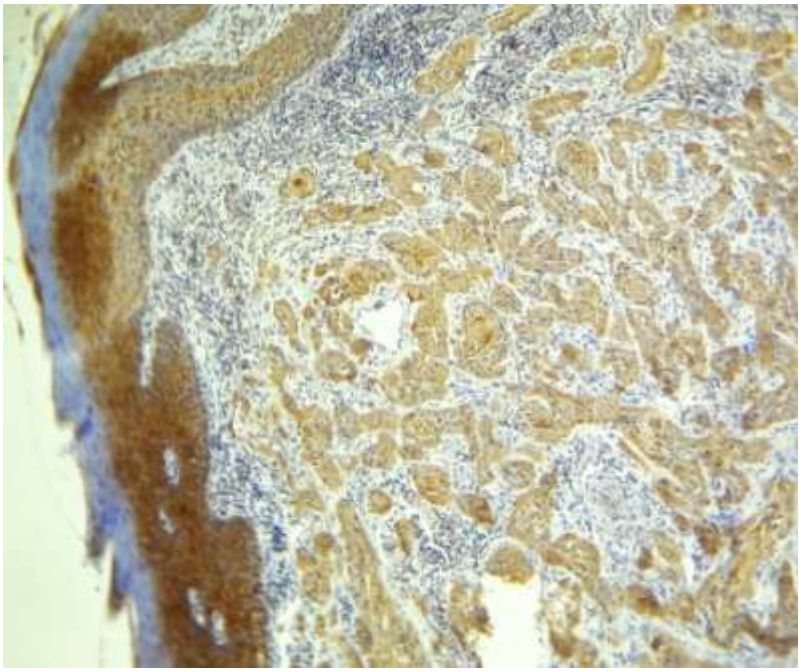


FIGURE 11 : MODERATELY DIFFERENTIATED SQUMAOUS CELL CARCINOMA – MASPIN SHOWING MODERATE EXPRESSION WITH STRONG POSITIVITY OF NORMAL SQUMAOUS EPITHELIUM (INTERNAL CONTROL)

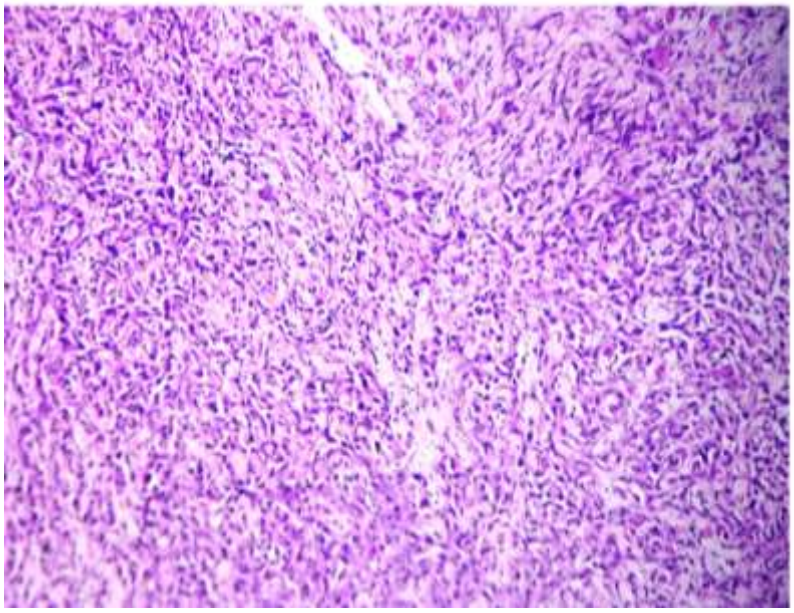


FIGURE 12 : POORLY DIFFERENTIATED SQUAMOUS CELL CARCINOMA

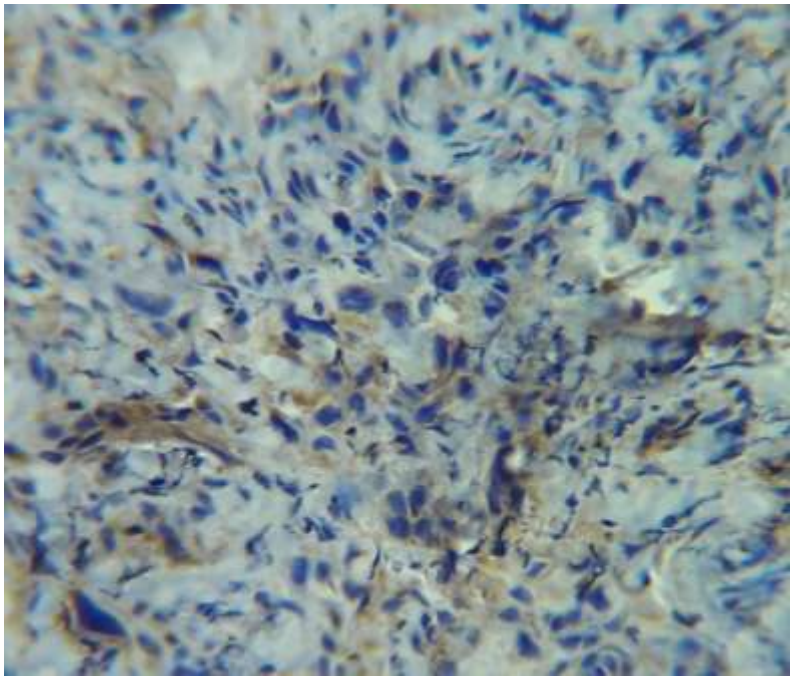


FIGURE 13 :POORLY DIFFERENTIATED SQUAMOUS CELL CARCINOMA SHOWING NEGATIVE STAINING FOR MASPIN

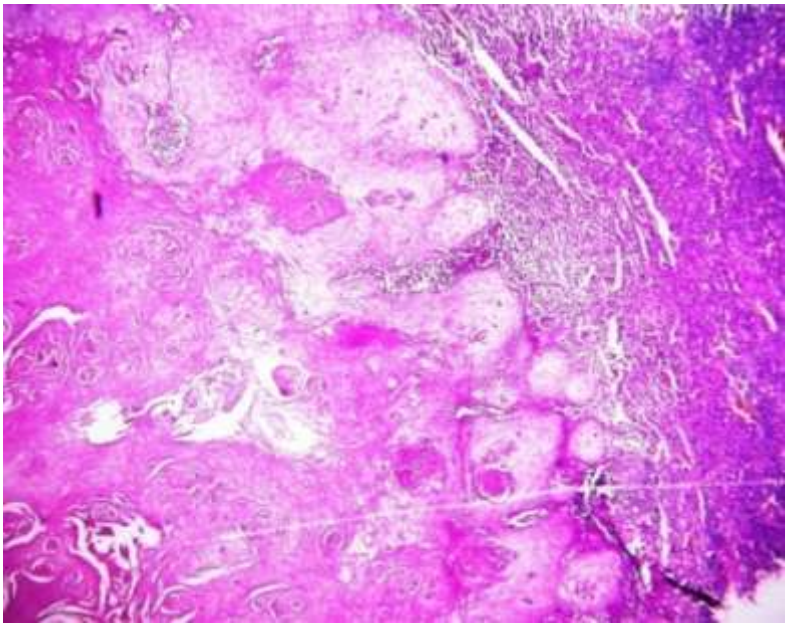


FIGURE 14 : LYMPH NODE SHOWING SQUAMOUS CELL CARCINOMA DEPOSITS

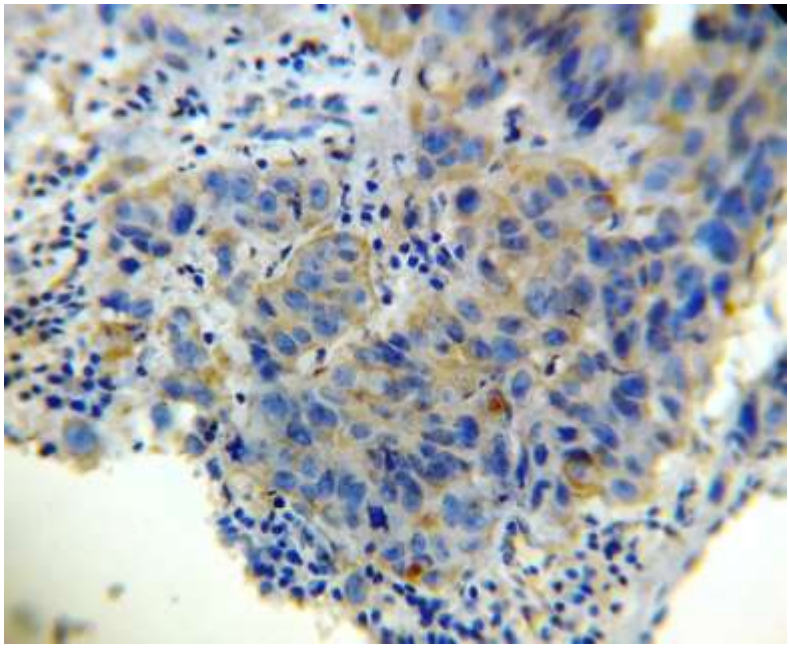


FIGURE 15 : WELL DIFFERENTIATED SQUAMOUS CELL CARCINOMA WITH NODAL METASTASIS SHOWING WEAK EXPRESSION OF MASPIN

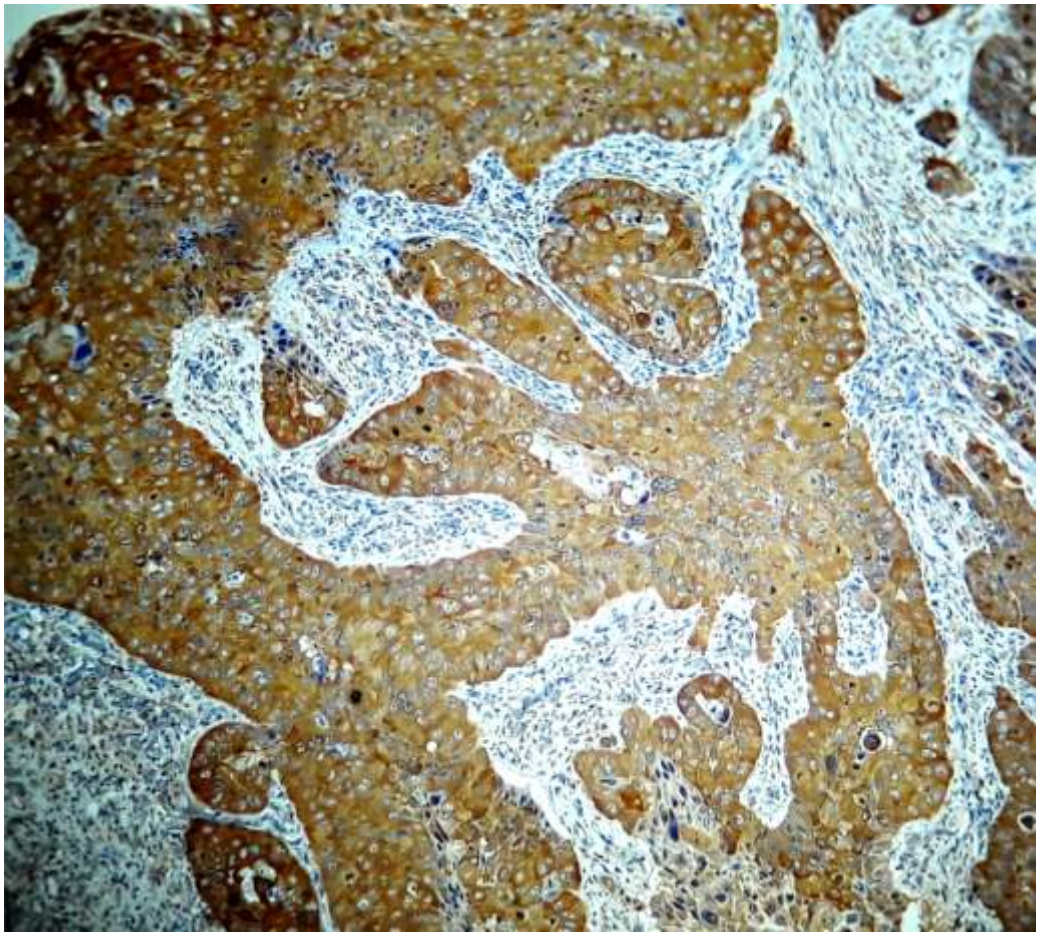


FIGURE 16 : MODERATELY DIFFERENTIATED SQUAMOUS CELL CARCINOMA WITH ABSENT NODAL METASTASIS SHOWING STRONG POSITIVITY FOR MASPIN

DISCUSSION

In Head and Neck Tumours , Squamous Cell Carcinoma remains the most common malignancy and it ranks 6th among the most common malignancy world wide.

In India the incidence of oral SCC is 30-50% of all malignancies. It is the commonest malignancy which has great impact not only on survival but also on the quality of life. Oral SCC has high potential for invasion into local tissues and also presents with high incidence of lymph nodal metastasis.

These malignancies are usually diagnosed at advanced stage and the expected 5 year survival rate falls to 10-40%. This poor survival rate is due to persistent uncontrollable disease and poor understanding at the molecular level.

Tumour metastasis is a multistep process includes local invasion , angiogenesis , disruption of adhesion to neighbouring cells and the extracellular matrix adhesion and transgression of endothelial cells to access into and out of the vascular circulation , attachment and proliferation at distant sites.

Maspin inhibits motility and cell invasiveness .It is considered that maspin is involved in inhibiting the initial step in the metastasis of oral squamous cell carcinoma.

This study aims at understanding the neoplastic transformation , progression and metastasis at molecular level . So novel therapeutic medication could be designed for treating head and neck squamous cell carcinoma.

In the current study , clinicopathological and immunohistochemical evaluation was done in 88 cases of SCC. An attempt was made to assess the significance of maspin expression and its correlation with prognosis , so that targeted therapy can be tried for better prognosis.

The total number of specimens received in Govt.Stanley Medical College from January 2016 to December 2016 was 9374 out of which 246 from oral cavity among that 153 cases were malignant.

AGE DISTRIBUTION OF OSCC:

This study showed that incidence of OSCC is from 3^{th} to 7 th decade . The highest incidence was noted in 5^{th} decade of life . The mean age of presentation was 55 . Other study Samina zaheer et al showed that mean age of presentation is 56.84 with a range falling between 5^{th} to 6^{th} decade.

	Samina zaheer et al	**Current study**
Mean age of presentation	56.84	53.55

SEX DISTRIBUTION :

Current study showed a male predominance of 82.95% compared with females which was about 17.05%. Comparative study of Samina zaheer et also showed an increased incidence among males than females.

SITE DISTRIBUTION:

In the current study the most common site was tongue with 39.59% followed by buccal mucosa and lip . In the study conducted by Samina Zaheer et al the incidence accounted to 32% in the tongue ,followed by 22% in the lip and 20% in the buccal mucosa.This was in accordance to the present study

Another study conducted by Yoshizawa et al showed that the common site was tongue accounting to 34%, lip being 20 % and buccal mucosa being 8%.

Comparision of distribution of oral SCC with other study groups :

Tumour site	**Samina Zaheer et al**	**Yoshizawa et al**	**Current study**
Tongue	32%	34%	39.59%
Buccal mucosa	20%	8%	23.88%
Lip	22%	20%	6.82%

In our study population , out of 88 cases , 36 cases were reported as well differentiated squamous cell carcinoma(fig 8), 36 cases were reported as moderately differentiated

squamous cell carcinoma(fig 10) and the remaining 16 cases were reported as poorly differentiated squamous cell carcinoma(12). When compared with other studies ,the following were the observation .

Histological grade of differentiation	**Samina zaheer et al**	**Yoshizawa et al**	**Current study**
Well differentiated	29	44	36(40.91 %)
Moderately differentiated	12	16	36 (40.91%)
Poorly differentiated	9	11	16 (18.18%)

WHO has classified the Squamous Cell Carcinoma into three grades based on the histopathological features, which includes both architectural and cytological criteria . In our study,both well differentiated and moderately differentiated SCC were reported in equal propotion .This was not in concurrence with other studies where well differentiated carcinoma was the most common type.

NODAL STATUS :

Nodal status	Xia et al	Yoshizawa et al	Current study
Nodal Metastasis Present	10	41	32(36.36 %)
Absent nodal metastasis	34	30	56(63.64 %)

In our study population out of 88 cases 56 (63.64 %) cases presented without nodal metastasis and remaining 32 cases presented with nodal metastasis. This was found going hand in hand with Xia et al where most cases were nodal negative.

TUMOUR STAGING :

Tumour staging	Yoshizawa et al	Current study
T1	19	43
T2	31	39
T3	6	6
T4	15	0

In our study population out of 88 cases , 43 cases presented at T1 stage ,39 cases were of T2 stage , 6 cases were of T3 and none of the cases presented in T4 stage. Thus in our study majority of cases were of T1 stage. This was not in concurrence with other study were T2 was the most common stage of presentation.

ASSOCIATION OF OVERALL MASPIN EXPRESSION AND HISTOLOGICAL GRADING OF TUMOUR :

Histological grade	Samina Zaheer et al	Yoshizawa et al	Current study
Well differentiated SCC	22	31	29
Moderately differentiated SCC	10	10	26
Poorly differentiated SCC	3	5	5

The overall maspin expression in any form such as strong , moderate and weak was taken as positive and such positivity was documented in 29 cases of well differentiated SCC, 26 cases of moderately differentiated SCC and 5 cases of poorly differentiated SCC in our study population.Thus it was observed that ,well differentiated carcinoma shows increased expression of maspin than pooly differentiated carcinoma . This was going hand in hand with Samina Zaheer et al andYyoshizawa et al. It shows statistically significant outcome with p value of 0.001 (table : 11)

COMPARISION OF ASSOCIATION OF OVERALL MASPIN EXPRESSION AND TUMOUR STAGING WITH OTHER STUDIES :

TUMOUR STAGING	YOSHIZAWA ET AL	CURRENT STUDY
I	100%	70.25%
II	100%	75.72%
III	80%	38.093%
IVA	66.7%%	8.333%
IV B	10%	0%

In our study population , overall Maspin expression was highest in stage I and stage II tumours . as stage progesses the expressionwas found to be decreased. This was in concurrence with Yoshizawa et al where the expression were reduced as the stage progresses.This shows a statistically significant outcome with p value of <0.001 (table 13).

COMPARISION OF OVERALL MASPIN EXPRESSION (OME) IN TUMOUR PRESENTS WITH LYMPHNODAL METASTASIS :

OVERALL MASPIN EXPRESION(OME)	XIA ET AL	YOSHIZAWA ET AL	CURRENT STUDY
POSITIVE	0	18(43.9%)	8(25%)
NEGATIVE	10	23(56.1%)	24(75%)

In our study population out 88 cases , 32 cases presented with nodal metastasis. The overall Maspin expression was positive in 25 % of cases and 75 % of cases shows negative Maspin expression. This was in concurrence with yoshizawa et al were 56.1 % cases shows negative Maspin expression and xia et al shows 100 % negative staining.

COMPARISION OVERALL MASPIN EXPRESSION(OME) IN TUMOUR WITH ABSENT LYMPHNODAL METASTASIS :

OVEALL MASPIN EXPESSION(OME)	XIA ET AL	YOSHIZAWAET AL	CURRENT STUDY
POSITIVE	15	2(6.7%)	4(7.142%)
NEGATIVE	19	28(93.3%)	52(92.58%)

In our study population out of 88 cases,56 cases presented without nodal metastasis. The overall Maspin expression was positive only in 4 cases (7.142 %) and 52 cases(92.58 %) showed negative Maspin expression. This was in concurrence with the study conducted by Yoshizawa et al which showed negative Maspin expression in 93.3

% cases. This showed a statistically significant out come with p value of < 0.001 in this study. The p value of our study is compared with other studies in the following table.

COMPARISION OF P VALUES WITH OTHER STUDY GROUPS:

STUDY GROUPS	PVALUES
XIA ET AL	0.009
YOSHIZAWA ET AL	<0.0001
CURRENT STUDY	**<0.001**

SUMMARY

- This study was a retrospective and comparative study conducted in the Department Of Pathology, Govt .Stanley Medical College during the period of January 2016 to December 2016.

- The total number of specimens received in Govt.Stanley Medical College were 9374 out of which 246 were from oral cavity and upper respiratory tract.

- 153 cases were documented as malignant out of the 9374 cases.

- Most common age group of occurrence of OSCC was noted in the 5^{th} decade with mean age of presentation at 53.55yrs.

- Males were commonly affected by OSCC with an incidence of 82.95% than females which accounted to 17.05%.

- Tongue was the most common site for oral SCC accounting to 39.59% followed by buccal mucosa accounting to 23.86 %.

- In this study we noted that Well differentiated SCC (36 cases)and Moderately differentiated SCC (36 cases) were reported in equal proportion and Poorly differentiated SCC in 16 cases.

- In this study 36.36% of cases presented with nodal metastasis and 63.64% of cases presented without nodal metastasis.

- In our study Oral SCC presented predominantly as stage I with an incidence of 36.36%.

- Among Well Differentiated SCC , Maspin was strongly expressed in 50% of cases and weakly expressed in 19.44% of cases .The intensity of staining score was score 3 in 27.7% of cases and score 0 in 8.33% of cases.

- In Moderately Differentiated SCC , Maspin was strongly expressed in 13.88% of cases and was negative in 27.77% of cases. The intensity of staining score was score 3 in 13.88 % of cases and score 0 in 5.555% of cases.

- In Poorly Differentiated SCC, none of the cases showed strong expression and

- 18.75 % of cases showed weak expression while 68.75 % of cases showed negative expression.The intensity of staining score 3 was not seen in any of the cases , score 1 was in 50 % of cases and score 0 in 43.75%.cases..

- Cases with absent nodal metastasis showed strong maspin expression accounting to 41.07 % and negative in 7.142 %.

- None of the Cases with nodal metastasis showed strong maspin expression while

- 75 % of cases showed negative expression .

- Thus in this study the overall maspin expression was highest in well differentiated squamous cell carcinoma and lowest in poorly differentiated squamous cell

carcinoma.Overall maspin expression was highest in nodal negative cases than nodal positive cases. Nodal positive cases showed a deceased maspin expression.

- Thus Maspin is considered as a prognostic marker, where loss of its expression indicates a bad prognosis with increased incidence of metastatic potential of such tumours .

CONCLUSION

This study showed the increased prevalence of Oral SCC among the general population .

Because of its aggressive nature , invasiveness and metastatic potential and its increasing trend in younger individuals , it is necessary to understand the carcinogenesis at molecular level.

In this study we attempted to evaluate the correlation between Maspin expression and various prognostic factors such as stage and grades of tumours . This study also evaluates the level of maspin expression in tumours presented with and without nodal metastasis.

This study showed a statistically significant association in the form of inverse correlation between the subcellular localization, histological grades ,stages of tumour and also with lymphnodal status.

However because of non availability of patient follow up details and outcome, the current study does not provide the idea about the Maspin expression with patient outcome and treatment effect.

Thus in future maspin can be consider as a prognostic marker for early diagnosis of metastasis and its potential for progression of the tumor. Since it is a prognostic marker it can be considered for targeted therapy for a better survival and outcome of the patients with SCC.

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MASTER CHART

S NO	BIOPSY NO	AGE	SEX	SITE	SIZE	DEPTH	T	N	GRADE	STAGE	NUMBER OF CELLS STAINED +INTENSITY OF STAINING	LEVEL OF EXPRESSION
1.	184/16	58	M	VOCAL CORD	1.5X1	1.5	T_1	N_0	G-I	I	3+3	STRONG
2.	341/16	48	M	BUCCAL MUCOSA	3X2.5	2	T_2	N_1	G-II	III	0+2	NEGATIVE
3.	353/16	56	M	OROPHARYNX	2.5X2	1	T_2	N_0	G-II	II	2+2	MODERATE
4.	399/16	75	M	TONGUE ANTERIOR 2/3	3X2	1	T2	N2	G-II	IV A	0+1	NEGATIVE
5.	441/16	65	M	LIP	2.5X2.5	2	T_2	N_1	G-I	III	2+1	MODERATE
6.	530/16	60	M	TONGUE ANTERIOR 2/3	3.5X2	1.5	T_2	N_2	G-I	IV A	0+1	NEGATIVE
7.	554/16	45	F	TONGUE ANTERIOR 2/3	0.5X0.5	0.5	T_1	N_0	G-I	I	3+2	STRONG
8.	573/16	42	M	TONGUE ANTERIOR 2/3	1.5X1	1	T_1	N_0	G-I	I	2+2	MODERATE
9.	686/16	62	M	LIP	1.5X1	0.5	T1	N_0	G-II	I	2+2	MODERATE
10	704/16	5	M	HYPO PHARYNX	2X1.5	1	T_1	N_0	G-II	I	2+1	MODERATE
11	733/16	67	M	TONGUE ANTERIOR 2/3	2.2X2	1	T_1	N_1	G-II	III	0+1	NEGATIVE
12	761/16	68	M	RETROMOLAR TRIGONE	3.5X2	1.5	T_2	N_1	G-II	III	0+1	WEAK
13	762/16	43	M	TONGUE ANTERIOR 2/3	3X3	1	T_2	N_1	G-II	III	0+1	NEGATIVE
14	776/16	64	M	BUCCAL MUCOSA	2X1	0.5	T_2	N_0	G-I	II	1+1	WEAK
15	879/16	50	M	SUPRAGLOTTIS	1X0.5	0.5	T_1	N_1	G-II	III	1+1	WEAK
16	884/16	47	M	HYPO PHARYNX	1X0.5	0.5	T_1	N_0	G-I	I	3+2	STRONG
17	985/16	40	M	HYPO PHARYNX	1X0.5	0.5	T_1	N_0	G-II	I	3+3	STRONG
18	1064/16	37	M	TONGUE ANTERIOR 2/3	2X1	1	T_2	N_0	G-I	II	2+3	STRONG
19	1091/16	45	M	TONGUE ANTERIOR 2/3	3X2	1	T_2	N_0	G-I	II	2+2	MODERATE
20	1092/16	62	M	VOCAL CORD	1X0.5	0.5	T_1	N_0	G-II	I	2+3	STRONG
21	1131/16	47	M	TONGUE ANTERIOR 2/3	2X1	0.5	T_1	N_0	G-I	I	3+2	STRONG
22	1317/16	50	F	TONGUE ANTERIOR 2/3	1.5X1	1	T_1	N_0	G-II	I	3+2	STRONG
23	1320/16	60	M	LARYNX SUPRA GLOTTIC GROWTH	1X0.5	0.5	T_1	N_1	G-III	III	0+1	NEGATIVE
24	1347/16	53	M	RETROMOLAR TRIGONE	3X2	1	T_1	N_3	G-II	IV B	0+3	NEGATIVE
25	1359/16	64	M	BUCCAL MUCOSA	2X1	1	T_1	N1	G-III	III	0+1	NEGATIVE
26	1360/16	55	M	OROPHAYNX	1.5X1	0.5	T1	N1	G-III	III	0+2	NEGATIVE
27	1364/16	48	F	HYPO PHAYNX	1X0.5	0.5	T1	N0	G-III	I	1+1	WEAK
28	1380/16	44	M	BUCCOL	3X3	2	T2	N2a	G-I	IV A	0+1	NEGATIVE

S NO	BIOPSY NO	AGE	SEX	SITE	SIZE	DEPTH	T	N	GRADE	STAGE	NUMBER OF CELLS STAINED +INTENSITY OF STAINING	LEVEL OF EXPRESSION
				MUCOSA								
29	1432/16	47	M	TONGUE	1.5X0.5 X0.5	0.5	T1	N2b	G-III	IV A	0+0	NEGATIVE
30	1450/16	70	M	CHEEK	4.2X	0.5	T3	N2b	G-III	IV A	0+0	NEGATIVE
31	1463/16	60	M	TONGUE ANTERIOR 2/3	1X0.5	0.5	T1	N2a	G-III	IV A	0+0	NEGATIVE
32	1541/16	53	M	BUCCAL MUCOSA	3X2X1	1	T2	N0	G-II	II	2+2	MODERATE
33	1619/16	50	M	HYPOPHARYNX	2.5X1	0.5	T2	N0	G-II	I	2+2	MODERATE
34	1712/16	50	M	TONGUE ANTERIOR 2/3	4.2X2.5X1.5	1.5	T3	N0	G-II	III	2+2	MODERATE
35	1770/16	60	M	BUCCAL MUCOSA	3.5X2.5	2	T2	N2b	G-II	IV A	0+0	NEGATIVE
36	1774/16	50	M	LARYNX VOCAL CORD	3.5X2	1	T2	N2b	G-II	IV A	0+1	NEGATIVE
37	1777/16	42	F	LIP	1X2	0.5	T1	N0	G1	I	3+3	STRONG
38	1828/16	39	M	LIP	4X3	1	T3	N2b	G-I	IV A	0+0	WEAK
39	1870/16	42	F	LARYNX	2X1	0.5	T1	N0	G-I	I	2+3	STONG
40	1949/16	55	M	LIP	4.5X2	1	T3	N1	G-II	III	1+1	WEAK
41	1952/16	50	M	VOCAL CORD	3.5X2.5	1	T2	N0	G-I	II	2+2	MODERATE
42	1953/16	41	M	TONGUE ANTERIOR 2/3	2.5X1.5	0.5	T2	N0	G-I	II	3+2	STONG
43	2052/16	57	M	BUCCAL MUCOSA	2X1	1	T1	NO	G-I	I	3+2	STRONG
44	2067/16	49	M	TONGUE ANTERIOR 2/3	3X2	1	T2	N0	G-II	II	2+2	MODERATE
45	2068/16	50	,F	LIP	2X1	0.5	T2	N0	G-I	II	2+2	MODERTAE
46	2069/16	55	F	EPIGLOTTIS	1.5X1	0.5	T1	N0	G- I	I	3+3	STRONG
47	2087/16	42	M	BUCCAL MUCOSA	2X1	1	T2	N0	G-I	II	3+2	STRONG
48	2191/16	35	M	GLOTTIS	2X1	0.5	T1	N0	G-III	I	3+1	MODERATE
49	2247/16	68	M	HYPO PHARYNX GROWTH	1.5X1	0.5	T1	N0	G-III	I	1+1	WEAK
50	2337/16	60	M	TONGUE ANTERIOR 2/3	2X1	0.5	T1	N0	GIII	1	2+1	MODERATE
51	2411/16	45	M	TONGUE	3X2	0.5	T2	N0	G-I	II	2+3	STONG
52	2443/16	68	M	BUCCOL MUCOSA	3.5X1	0.5	T2	N0	G-I	II	3+3	STONG
53	2538/16	59	F	HYPO PHARYNX	2X1.5	1	T2	N0	G-II	I	2+1	MODEATE
54	2614/16	58	M	HYPOPHARYNX	1.5X1	0.5	T1	N0	G-II	I	2+1	MODERATE
55	2654/16	55	M	LARYNX	2.5X1.5	1	T2	N0	G-II	II	2+1	MODERATE
56	2815/16	65	M	LARYNX	305X2	1	T2	N1	G-II	III	1+1	WEAK
57	2826/16	40	M	TONGUE ANTERIOR 2/3	3X2	1	T2	N1b	G-II	III	0+1	NEGATIVE
58	2850/16	70	F	ORAL CAVITY	2X1	0.5	T2	N0	G-II	II	2+2	MODERATE
59	2853/16	60	M	LARYNX	1.5X1	0.5	T1	N0	G-II	I	2+2	MODERATE
60	2928/16	47	M	HYPO PHARYNX	1.5X1	0.5	T1	N0	G-I	I	3+3	STRONG
61	3949/16	55	M	BUCCAL MUCOSA	1.5X1	0.5	T1	N2b	G-III	IVA	0+O	NEGATIVE
62	3048/16	41	F	TONGUE	2.5X2	2	T2	N0	G-I	II	0+2	NEGATIVE

S NO	BIOPSY NO	AGE	SEX	SITE	SIZE	DEPTH	T	N	GRADE	STAGE	NUMBER OF CELLS STAINED +INTENSITY OF STAINING	LEVEL OF EXPRESSION
				ANTERIOR 2/3								
63	3566/15	45	M	TONGUE ANTERIOR 2/3	2.5X2	1	T2	N1	G-I	III	0+2	NEGATIVE
64	2920/16	55	M	CHEEK	2X1	0.5	T1	N0	G-I	I	3+3	STRONG
65	2443/16	68	M	HARD PALATE	3.5x2	1	T2	N1	G-I	III	1+1	WEAK
66	2411/16	56	M	TONGUE ANTERIO 2/3	1.2x0.5	0.5	T1	N0	G-I	I	3+2	STRONG
67	3828/15	50	F	CHEEK	2x1	1	T1	N0	G-I	I	3+3	STRONG
68	776/16	54	M	BUCCAL MUCOSA	4x3.5	1	T2	NO	G-I	II	2+2	MODERATE
69	2357/15	53	M	BUCCAL MUCOSA	1x1	0.5	T1	NI	G-I	III	1+1	WEAK
70	6434/15	40	M	BUCCAL MUCOSA	2.7x1	0.5	T2	N2B	G-I	IVA	0+0	NEGATIVE
71	3471/16	40	F	TONGUE ANTERIOR2/3 (4)	3x2	1	T2	N0	G-I	II	1+1	WEAK
72	6389/15	70	M	RETRO MOLAR TRIGONE (12)	2x2	1	T1	N0	G1	I	3+2	STRONG
73	2671/15	47	M	POSTERIOR 1/3 TONGUE	2.5x1	0.5	T2	N1A	G1	III	0+1	NEGATIVE
74	2850/16	70	F	BUCCOL MUCOSA	1x1	0.5	T1	N2	G1	IVA	0+0	NEGATIVE
75	3809/15	65	F	TONGUE ANTERIOR 2/3	0.75x0.75	0.5	T1	N0	G-II	I	3+3	MODERATE
76	4099/15	65	M	POST 1/3 TONGUE	1x0.5	0.5	T1	N0	G-II	I	3+3	STRONG
77	6528/15	35	M	BUCCAL MUCOSA	3.5x2	1	T2	N0	G-II	II	2+2	MODERATE
78	6435/15	72	M	RIGHT BUCCAL MUCOSA	3x2	1	T2	NO	G-II	II	2+2	MODERATE
79	3785/15	32	M	ANTERIOR 2/3TONGUE	1x1	0.5	T1	N0	G-II	I	3+2	STRONG
80	2231/15	55	M	BUCCAL MUCOSA	4.5x3	1.5	T3	N1A	G-II	III	0+0	NEGATIVE
81	991/15 A	55	M	ANTERIOR 2/3RDTONGUE	3x3	1.2	T2	N1A	G-II	III	0+2	NEGATIVE
82	3466 A	60	M	PYRIFORM FOSSA GROWTH	1x0.5	0.3	T1	N0	G-II	I	2+1	MODERATE
83	3466 B	60	M	POSTERIOR 1/3 TONGUE GROWTH	1x0.75	03	T1	N0	G-II	I	1+1	WEAK
84	5871/15	63	M	TONGUE	3x1.2	0.75	T2	N0	G III	II	0+0	NEGATIVEE
85	2160/15	60	F	LIP	3.3x2	1	T2	N1	G III	III	0+0	NEAGTIVE
86	2191/16	35	M	RETROMOLAR TRIGONE	4.2x2	1	T3	N0	G III	III	0+0	NEAGTIVE
87	1320/16	60	M	VOCAL CORD	1x1	0.75	T1	N0	G III	I	1+1	WEAK
88	2494/16	55	M	OROPHARYNX	0.75x0.5	0.5	T1	N0	G III	I	0+0	NEGATIVE

.ANNEXURE – I

WHO CLASSIFICATION OF TUMOURS OF THE ORAL CAVITY AND OROPHARYNX

Malignant epithelial tumours Squamous cell carcinoma Verrucous carcinoma Basaloid squamous cell carcinoma Papillary squamous cell carcinoma Spindle cell carcinoma Acantholytic squamous cell carcinoma Adenosquamous carcinoma Carcinoma cuniculatum Lymphoepithelial carcinoma **Epithelial precursor lesions** **Benign epithelial tumours** Papillomas Squamous cell papilloma and verruca vulgaris Condyloma acuminatum Focal epithelial hyperplasia Granular cell tumour Keratoacanthoma **Salivary gland tumours** Salivary gland carcinomas Acinic cell carcinoma Mucoepidermoid carcinoma	Adenoid cystic carcinoma Polymorphous low-grade adenocarcinoma Basal cell adenocarcinoma Epithelial-myoepithelial carcinoma Clear cell carcinoma, not otherwise specified Canalicular adenoma Duct papilloma CystadenomaCystadenocarcinoma Mucinous adenocarcinoma Oncocytic carcinoma Salivary duct carcinoma Myoepithelial carcinoma Carcinoma ex pleomorphic adenoma Salivary gland adenomas Pleomorphic adenoma Myoepithelioma Basal cell adenoma **Soft tissue tumours** Kaposi sarcoma Lymphangioma Ectomesenchymal chondromyxoid tumour Focal oral mucinosis Congenital granular cell epulis

Haematolymphoid tumours

Diffuse large B-cell lymphoma (DLBCL)

Mantle cell lymphoma

Follicular lymphoma

Extranodal marginal zone B-cell lymphoma of MALT type

Burkitt lymphoma

T-cell lymphoma (including anaplastic large cell lymphoma

Extramedullary plasmacytoma

Langerhans cell histiocytosis

Extramedullary myeloid sarcoma

Follicular dendritic cell sarcoma / tumour

Mucosal malignant melanoma

Secondary tumours

ANNEXURE-II

WHO HISTOLOGICAL CLASSIFICATION OF TUMOURS OF THE NASOPHARYNX

Malignant epithelial tumours

Nasopharyngeal carcinoma

Nonkeratinizing carcinoma

Keratinizing squamous cell carcinoma

Basaloid squamous cell carcinoma

Nasopharyngeal papillary adenocarcinoma

Salivary gland-type carcinomas

Benign epithelial tumours

Hairy polyp

Schneiderian-type papilloma

Squamous papilloma

Ectopic pituitary adenoma

Salivary gland anlage tumour

Craniopharyngioma

Soft tissue neoplasms

Nasopharyngeal angiofibroma

Haematolymphoid tumours

Hodgkin lymphoma

Diffuse large B-cell lymphoma Extranodal NK/T cell lymphoma

Follicular dendritic cell sarcoma/tumour X

Extramedullary plasmacytoma

Tumours of bone and cartilage

Chordoma

Secondary tumours

ANNEXURE-III

WHO HISTOLOGICAL CLASSIFICATION OF TUMOURS OF THE HYPOPHARYNX, LARYNX AND TRACHEA

Malignant epithelial tumours	**Benign epithelial tumours**
Squamous cell carcinoma	Papilloma
Verrucous carcinoma	Papillomatosis
Basaloid squamous cell carcinoma	Salivary gland-type adenomas
Papillary squamous cell carcinoma	Pleomorphic adenoma
Spindle cell carcinoma	Oncocytic papillary cystadenoma
Acantholytic squamous cell carcinoma	**Soft tissue tumours**
	Malignant tumours
Adenosquamous carcinoma	Fibrosarcoma
Lymphoepithelial carcinoma	Malignant fibrous histiocytoma
Giant cell carcinoma	Liposarcoma
Malignant salivary gland-type tumours	Leiomyosarcoma
Mucoepidermoid carcinoma	Rhabdomyosarcoma
Adenoid cystic carcinoma	Angiosarcoma
Neuroendocrine tumours	Kaposi sarcoma
Typical carcinoid	Malignant peripheral nerve sheath tumour
Atypical carcinoid	
Small cell carcinoma, neuroendocrine type	Synovial sarcoma
	Borderline tumours / LMP
Combined small cell carcinoma, neuroendocrine type	Inflammatory myofibroblastic Tumour

Benign tumours

Schwannoma

Neurofibroma

Lipoma

Leiomyoma

Rhabdomyoma

Hemangioma

Lymphangioma

Granular cell tumour

Haematolymphoid tumours

Tumours of bone and cartilage

Chondrosarcoma

Osteosarcoma

Chondroma

Giant cell tumour

Mucosal malignant melanoma

Secondary tumours